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BABESIASIS.

(385) VRIJBURG (A.). **Einige Untersuchungen über** *Babesia bigemina*. [Some Experiments with *Babesia bigemina*.]—*Zeitschr. f. Infektionskrankh. parasit. Krankheit. u. Hygiene d. Haust.* 1913. Mar. Vol. 13. No. 3-4. pp. 180-186.

Attempts to cultivate Babesia bigemina *in vitro*.—

Blood was withdrawn from the jugular vein of a calf which was suffering from a relapse of the disease without haemoglobinuria. The blood was carefully defibrinated without frothing and 50 per cent. sterile solution of dextrose added in the proportion of 1 per cent. This mixture was then centrifuged.

After complete centrifugalisation the layer of serum was carefully pipetted off and distributed into tubes, the red corpuscles being then withdrawn by passing the pipette right to the bottom of the tube. The red corpuscles were ejected into the bottoms of the tubes in which the serum had been previously placed. According to BASS and JOHNS, who devised the method for the cultivation of the malaria parasite, red blood corpuscles alone can be obtained by this method, but the present author was unable to get red corpuscles quite free from leucocytes.

Tubes so prepared were incubated at 37° C. and 22° C. respectively.

Subcultures were made on the third day, the blood mixture being prepared in the same way as before save that blood from a healthy animal was used. Blood corpuscles were withdrawn from the primary tubes and added to the fresh tubes, the quantity added being about a fifth of the volume. These were also incubated at 37° C. and 22° C.

After a further three days a third series of tubes was inoculated from the second. Smear preparations were examined from the different tubes at intervals.

In each smear made from the primary cultures incubated at both temperatures two parasites were found on an average, while none were found in control tubes containing simple defibrinated blood. On the sixth and ninth days no parasites were discoverable in the primary cultures.

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No parasites could be found in the cultures of the second generation incubated at 37° C. In a preparation made on the third day from a tube incubated at 22° C. six parasites were found, but none were found on the sixth and ninth days.

No parasites were found in preparations from the third generation of cultures.

In a second series of experiments the dextrose was replaced by glucose, the preparation of the tubes and the incubation being the same as before.

In control tubes of defibrinated blood incubated at 37° and 22° C. no parasites were discoverable on the sixth day.

In the tubes of the first generation incubated at 37° C. parasites were just as numerous as in the blood, but there was no multiplication. A few days later all the parasites had disappeared.

Smear preparations from the tubes incubated at 22° C. showed that multiplication had occurred. Parasites, most of which were of the amoeboid type, were found both intra- and extra-corpuscular in position, and a very small number of double forms was encountered. A few days later some coccus-like bodies were found both within and external to the blood corpuscles. After twelve days no parasites could be found.

The tubes of the second generation showed about six parasites per smear on the third day, and after about the thirteenth day none could be found.

There was no multiplication in the tubes of the third generation incubated at 37° C.

In a preparation made from a tube incubated at 22° C. a number of rounded bodies measuring 1 to 2 microns in diameter were found, some within and some external to the blood corpuscles. These could not be found ten days later.

A further series of tubes incubated at 22° C. failed to show any multiplication of the parasites.

Attempts to cultivate the parasite in the condensation water of blood agar and of dextrose blood agar failed.

The small variety of Babesia bigemina.—

After giving a brief abstract of McFadyean and Stockman's description of *Bebesia divergens* the author states that during very frequent examinations of the blood of cattle suffering from haemoglobinuria in Holland he has only encountered small parasites. The majority of the organisms are either irregularly rounded or slender. Pear-shaped parasites are not found. The slender forms have a bluntly rounded end and a pointed end, and they vary in length from 1·5 to 2, and exceptionally 2·5 microns in length. The rounded forms have a maximum diameter of 1·5 microns.

Single parasites are more frequently observed in the blood corpuscles than double ones.

In the elongated forms there is a somewhat large mass of chromatin generally disposed at the blunt end, and in the rounded forms the amount of cytoplasm is usually very small.

BRITISH MUSEUM

Details are given of the microscopic examination of the blood of three infected animals and from these the following facts may be gathered:—

The percentage of infected corpuscles may be as high as 40. The rounded forms outnumber the slender forms. Very few parasites are to be found free in the plasma. A greater proportion of the parasites occur singly in the corpuscles, and of these about 50 per cent. are centrally and 50 per cent. peripherally placed. The percentage of single parasites lying at the rim of the corpuscles was 7 in one case and 51 in another. The percentage of double parasites varied from 16 to 30, and these in the great majority of cases did not lie towards the periphery of the corpuscles. In one instance 50 per cent. of these showed marked divergence, but in another it was observed in 3 per cent. only.

In a small proportion of cases three or even four parasites were encountered in a single corpuscle.

Animals infected experimentally with this parasite showed very slight reactions, in some instances there was only slight fever without parasites being discoverable in the blood.

The author concludes that the parasite which occurs in Holland resembles *B. divergens* save that the divergence of the double parasites is not usually observed and that the parasites do not show a special tendency to take up a position at the rim of the corpuscles. He thinks that the name *B. divergens* is not applicable to the parasite and that the organism is a small form of *B. bigemina* such as has been described as occurring in Germany and North Africa.

No cross-immunity experiments have been done, but the author suggests that should these indicate non-identity the name "*Babesia bovis*" should be given to the small parasite.

(386) Dschunkowsky (E.) & Luhs (T.). *Nuttallia* **und** *Piroplasma* **bei der Piroplasmose der Einhufer in Transkaukasien.** [*Nuttallia* and *Piroplasma* in Piroplasmosis of solipeds in Transcaucasia.] — *Parasitology.* 1913. Jan. Vol. 5. No. 4. pp. 289-302. With 2 plates.

In this paper all the cases of piroplasmosis that have been observed by the authors in solipeds are summarised, and the conclusion arrived at supports the view that two species of parasite are concerned—*Nuttallia* and *Piroplasma*.

The first case observed occurred in an imported horse which died as a result of the infection. Examination of the blood in this case showed that about 1 per cent. of the red cells contained parasites. The great majority of these were rounded in shape, but oval, pear-shaped, and amoeboid forms were seen. The authors think that the African parasite, *Nuttallia equi*, is differentiated from that observed in the first case in Transcaucasia by the fact that in the former amoeboid forms are the most commonly observed type of parasite, while the rounded parasites are far fewer in number. A further point of difference is the frequent occurrence of cross-forms in Nuttalia, this being the manner in which multiplication occurs in this species.

In mature parasites the chromatin mass is generally single and rounded in shape. It is practically impossible to make out a blepharoplast, but in some parasites the chromatin is present in the form of a longer or shorter curved rod in which darkly stained granules can be observed.

The cytoplasm stains blue, and has a reticulate structure. It appears to be more condensed towards the periphery of the parasite.

The chomatin is nearly always surrounded by a clear zone.

The authors mention a striking character possessed by the parasites studied by them, namely, a marked variation in size. The rounded forms varied from 0·8 to 2·8 microns in diameter, and the pear-shaped forms from 1·5 to 4 microns in length, by 1·0 to 1·5 microns in width.

They are of the opinion that the smallest parasites do not represent young forms but mature organisms, and, consequently, they think that they are not dealing with a single organism, but with two separate varieties—a large and a small. They are not at present in a position to say which parasites these represent.

The possibility is suggested that in Transcaucasia there are three distinct types of parasite—a large and a small *Nuttallia*, and *Piroplasma caballi*.

The cases of the disease observed in 1906 and 1907 were all due to parasites of the genus Nuttallia save one which was caused by Piroplasma.

In some cases of Nuttallia infection the percentage of infected corpuscles rose as high as 30 to 40.

At the post-mortem of the horse in which there was a mixed infection the only lesions found were great enlargement of the spleen (up to 5 or 6 times) and yellow discoloration of the mucous and serous membranes. There were no abnormalities observable in the blood save the presence of parasites. The disease ran an acute course.

The piroplasms presented a typical pear-shaped form resembling the large parasites seen in bovine piroplasmosis. In a minority of cases two parasites were present in the same corpuscle, but more than two were not observed. The parasites were joined together by their thin ends, and in many cases there was a small round granule of chromatin at the point of junction.

The large parasites varied from 2·4 to 3·7 microns in length and from 1·0 to 1·5 in width. In practically all the pear forms a nucleus and blepharoplast could be made out.

Some of the pear-shaped or oval parasites showed bud-like processes which appeared to suggest that the parasites were dividing. There were two bud-like processes in every instance and in the great majority of cases they were placed on one side of the parasite. After their liberation from the parent cell these processes appeared to form twin pear-shaped parasites which afterwards separated from each other.

In some of the pear-shaped organisms the chromatin breaks up into a number of fragments which pass either to the periphery of the parasite or remain scattered through the cytoplasm.

The amoeboid forms which are always observed are distinguishable from the budding forms already described. They have

a very irregular outline and possess pseudopodia which are either pointed or thickened at the ends.

The forms already described have been observed in blood taken during life.

In the blood of an animal which had been dead five hours only round parasites were seen which had a shrunken appearance.

With regard to the pear-shaped parasites of the two genera, the authors make the following statements:—

In *Nuttallia* pear forms are far more scanty than round forms. In Piroplasma the pear form is by far the most common and characteristic. In *Nuttallia* pear forms in most cases occur singly in the corpuscles, and when two are present in a single cell they are not united by their thin ends. In *Piroplasma* double pear forms commonly occur and these are generally joined together. In *Nuttallia* the nucleus and the blepharoplast are practically always together, while in Piroplasma they are quite easily distinguishable from each other. Further, in the former the nucleus is practically always round while in the latter it is often elongated or fragmented. The structure of *Piroplasma* appears to be more complicated than that of *Nuttallia* owing to the presence of vacuoles in the cytoplasm and to the fragmentation of the chromatin.

In all, twelve cases of the disease have been observed, and in only one of these was *Piroplasma* found and then in conjunction with *Nuttallia*.

In one instance infection with *Nuttallia* was observed in a mule. Two donkeys and one horse were inoculated with large doses (500, 100, and 800 c.c.) of defibrinated blood. In one animal only, the donkey which received 100 c.c., was there any reaction, and parasites were found in the blood on the thirteenth day.

In order to gain some idea as to the prevalence of the disease among donkeys, 18 animals which were unfit for work were examined, and eight were found to be infected. Eleven of the donkeys died in the course of two months, and four were found to have parasites in their blood. At the post-mortem examination no abnormalities save the lesions of anaemia were discovered. There was no swelling of the spleen.

Four of the infected donkeys survived.

The authors think that the parasite found in the donkeys is possibly a definite species of *Nuttallia*, but they do not make a definite pronouncement on the point.

(387) von Rátz (S.). **Ueber die Piroplasmose der Schafe.** [Piroplasmosis in the Sheep.]—*Centralbl. f. Bakt.* 1 Abt., Orig. 1913. Mar. 1. Vol. 68. No. 2. pp. 194-200. With 2 text-figures.

After reviewing the literature regarding the occurrence of piroplasmosis in the sheep, the author refers to an outbreak of disease in sheep in Hungary in 1909, in which piroplasms were discoverable in the blood. In view of the facts that the lesions found on post-mortem were not indicative of piroplasmosis, and

that inoculation experiments failed no great importance was attached to the discovery.

In the autumn of 1911 Balás found the following lesions in a sheep which had been slaughtered:—

Numerous punctiform haemorrhages in the subcutaneous connective tissue. Anaemia of all the organs and a laked condition of the blood. A small number of haemorrhages in the serous membranes, and slight swelling of the spleen. Catarrh of the mucous membrane of the large and small intestines associated with punctiform haemorrhages, particularly in the region of the pylorus. Numerous haemorrhagic infarcts of various sizes in the lungs. Acute swelling of the lymphatic glands.

The spleen was forwarded for examination, and in smears stained by the May-Grunwald method piroplasms were discovered in considerable numbers.

There were practically no abnormalities to be observed in the red corpuscles, and they took the stain fairly well. Only those cells which contained from 1 to 3 large parasites showed any enlargement.

The parasites were very variable in size and shape. They measured from 9·8 to 2·0 microns in diameter, and rounded, oval, rod-shaped, and ring-forms were seen. In the majority of cases the parasites were surrounded by a lighter zone. Save in a few instances they were placed peripherally in the infected corpuscles. The smallest of the parasites resembled cocci, but they showed in their centre a minute nucleus which stained of a bluish red tint. The larger round parasites presented a similar appearance, but their nuclei were proportionately larger. In some cases they appeared to be dividing longitudinally, and in each portion there was a piece of chromatin. When pairs of small parasites occurred they presented an appearance suggestive of diplococci.

Oval forms were not uncommon, and in these the nucleus was situated at or near one of the poles. In the majority of cases these parasites occured singly in corpuscles, but in some cases the same cell contained either mono- or diplococcus forms in addition. Some of the round or oval forms possessed irregular pseudopodia. Short thick rod-like forms occurred in small numbers and in these there was a piece of chromatin at each end. Curved rod forms were observed applied to the outer surface of red corpuscles.

The most characteristic forms were the pear-shaped organisms which occurred singly or in pairs. In the centre of these larger parasites there was a distinct nucleus, and in many of them a small fragment of chromatin, possibly representing a blepharoplast, could be made out at one end. When two pear-shaped parasites occurred in a single corpuscle they were as a rule smaller than the single ones. The relative positions occupied by the two parasites varied greatly. In some cases they were nearly parallel to each other and in others they formed a wide angle or even a straight line.

A few ring forms were observed. In these the central part appeared vacuolar, and a rounded or elongated nucleus could be seen in the cytoplasmic rim.

In a few large corpuscles, which did not stain intensely, rosette forms of reddish granules could be seen. In many of these no cytoplasm could be distinguished.

Parasites at different stages of development could be found in a single corpuscle, and very few parasites were seen free in the plasma.

In the second outbreak of the disease a number of lambs were attacked, the ewes and the very young lambs escaping infection.

The lesions found at the post-mortem of one of these animals did not suggest piroplasmosis, but the parasite was discovered in the blood. The principal lesions were: Enlargement of the lymphatic glands, parenchymatous degeneration of the kidneys, liver, and cardiac muscle, sero-fibrinous pericarditis and haemorrhages under the epicardium.

The parasites present in the blood exactly resembled those seen in the previous case, save that the rounded and amoeboid forms were the most numerous.

In some of the pear-shaped parasites indications of division could be observed.

Subsequently the author found piroplasms in smears from three spleens which were forwarded for examination.

It would appear that two forms of the disease occur, an acute and fatal form, and a mild and chronic or latent form. In many cases the lesions are so little characteristic that only examination of the blood allows a diagnosis to be made.

(388) Knuth & Richters. **Uber die Vermehrung von *Piroplasma canis* in vitro.** [The Multiplication of *P. canis* in vitro.]—*Berl. Tierärzt. Wochenschr.* 1913. Mar. 20. Vol. 29. No. 12. pp. 211-212.

The authors have not strictly followed the technique of Bass as did Ziemann in his successful cultivation of *P. canis*.

In the original tube blood containing a few parasites was mixed with one per cent. dextrose solution in the proportion of two to one. The mixture was defibrinated and freed from leucocytes by centrifugalisation. The tube was then incubated at 40° C. About ¼-½ cc. of this mixture was then added to a tube containing 8 cc. of normal dog blood to which 4 cc. of one per cent. dextrose solution had been added. This mixture was also defibrinated, and centrifuged for the removal of the leucocytes. In both of these tubes there was a multiplication of the parasites in 18 to 20 hours.

In subsequent experiments the serum was not removed and a two or even three per cent. solution of dextrose was substituted for the one per cent. solution originally used. Some of the tubes were incubated at 40-41° C., some at 37·5° C., and some were left at room temperature.

The best results were obtained in those tubes which contained a mixture of defibrinated blood and a two per cent. solution of dextrose and which were left at room temperature. In these tubes there was a distinct multiplication of the parasites in eight days.

THEILERIASIS.

(389) Carpano (M.). **Su di un Piroplasma del tipo Parvum (genus *Theileria*) riscontrato nella Gazzella in Eritrea. Nota di priorità.** [A Piroplasm of the Parvum type (genus *Theileria*) in a Gazelle in Eritrea.] — *La Clinica Veterinaria.* 1913. Mar. 30. Vol. 36. No. 6. pp. 254-256.

In 1909 while travelling in Eritrea the author saw a gazelle infected with a piroplasm of the parvum type. The animal was in good condition, and all its organs including the spleen were normal in appearance. The parasite was not very numerously present in the blood, about two infected corpuscles being found in each field of the microscope (1/15 inch objective and ocular 6).

In the great majority of cases each infected corpuscle contained a single parasite only, and corpuscles containing more than two were exceedingly rare.

The following forms were observed:—

Small rounded forms which appeared to be composed almost entirely of chromatin.

Round or oval ring forms. These stained blue, and showed at one side two masses of chromatin, which were either rounded, curved, or ring-shaped.

Pear or club-shaped forms. These, like the preceding, measured from one to one and a half microns in diameter. The masses of chromatin in the majority of instances were placed at the thinner end of the parasite.

Comma forms. In these the protoplasm was coloured throughout and the mass of chromatin was either round or elongated.

Bacillary forms. These measured from 2 to 3 microns in length. In these also the chromatin was either rounded or elongated in shape.

The comma forms and the bacillary forms were the most numerous, then the oval and round forms. In cases in which four parasites were present in a single corpuscle they were arranged in the form of a cross.

TRYPANOSOMIASIS.

(390) Bruce (D.), Harvey (D.), Hamerton (A. E.), Davey (J. B.), & Lady Bruce. **The Trypanosomes found in the Blood of Wild Animals Living in the Sleeping-Sickness Area, Nyasaland.** —*Proc. Roy. Soc.* 1913. Apr. 7. Series B. Vol. 86. No. B 587. pp. 269-277.

In this paper the greater part of the information given is expressed in tabular form. The first table gives a list of 180 animals shot in the fly area on the shores of Lake Nyasa whose blood was examined for trypanosomes. In the great majority of cases the examination included the preparation of thick and thin smears, and the inoculation of goats, monkeys, and dogs. Of these 180 animals 57 or 31·7 per cent. were found to harbour pathogenic trypanosomes.

The second table gives the species of trypanosomes found in the various infected animals. With regard to *T. rhodesiense* the following paragraphs may be quoted verbatim.

"In a previous paper the trypanosome causing human trypanosome disease in Nyasaland was called *Trypanosoma rhodesiense*, on account of the presence of posterior-nuclear forms. This trypanosome agreed in all other respects with *Trypanosoma brucei*, the common trypanosome of wild animals in South Africa, and the cause of tsetse-fly disease, or Nagana. In order to compare the two species of trypanosomes more closely, the Commission procured, by the kindness of Dr. A. Theiler, C.M.G., Pretoria, a strain of nagana from the same spot in Zululand where it was first discovered in 1894. Much to the surprise of the Commission it was found that *T. brucei* has quite as large a proportion of posterior-nuclear forms as *T. rhodesiense*, and that the blunt-ended character is common to both species. The Commission is therefore driven to the conclusion that *T. rhodesiense* is neither more nor less than *T. brucei*, and that the human trypanosome disease of Nyasaland is Nagana. To this it may be objected that Nagana has never been known to attack human beings. This has probably been due to faulty diagnosis, cases in man being returned as malaria."

It would appear from the tables given that among the 180 animals examined *T. rhodesiense* (vel *brucei*) was encountered 14 times, *T. pecorum* 26 times, *T. simiae* 3 times, *T. caprae* 20 times, and *T. ingens*, which is not considered to be pathogenic, 3 times. Although the numbers are too small to be taken literally it is shown that waterbuck, hartebeeste, reedbuck, and duiker are most dangerous as far as man is concerned; eland, koodoo, bushbuck, and buffalo to cattle, goats, and sheep; and that the warthog is the only animal that harbours *T. simiae*, "the lightning destroyer of the domestic pig."

No pathogenic trypanosome has as yet been discovered in the blood of animals living in fly-free areas.

(391) Bruce (D.), Harvey (D.), Hamerton (A. E.), & Lady Bruce. **Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland. I.—The Human Strain.** —*Proc. Roy. Soc.* 1913. Apr. 7. Series B. Vol. 86. No. B 587. pp. 285-302.

In this paper the morphology of four strains of the human trypanosome is described in great detail, the results of the measurements made being expressed in a number of tables and curves. In the tables are incorporated the measurements made of a fifth strain of the trypanosome (Strain I, Mkanyanga) which have already been published. (See this *Bulletin*, Vol. 1, No. 2, Ref. No. 204, p. 134.)

Comparison is instituted between the measurements and the curves obtained when the trypanosomes measured were in the blood of various animals (goat, monkey, dog, rat and sheep) and when the measurements were made in a systematic manner on consecutive days of the trypanosomes present in the blood of a single rat.

In the blood of various animals the average length of the five different strains varied from 22·2 to 26·1 microns with an average of 23·5, and in the blood of rats the lengths varied from 22·5 to 26·4, the strains falling nearly, but not quite, in the same order.

In dealing with one of the strains four curves are given. These curves were constructed as follows:—(1) upon 1,500 trypanosomes from various animals, (2) upon 1,000 parasites taken at random, (3) upon 500 trypanosomes taken from a rat on nine consecutive days, (4) as (3), but from a different rat. When these curves are compared it is seen that curves (1) and (2) differ considerably from each other and from (3) and (4) which are very much alike.

To quote the paper, "If curves made in this way from different strains of one species of trypanosome showed the same degree of similarity, this method would certainly be useful for purposes of classification. But, as we have seen, the curve of Strain II has no resemblance to that of Strain I (previous publication), and it will be found that each human strain of this species of trypanosome differs, more or less, when subjected to this method of measurement."

In comparing the curves constructed upon the whole of the five strains the authors express themselves as follows:—

"It must be confessed that, on comparing the five curves one with another, they do not give as much assistance in classifying this species of trypanosome as was hoped. Curves I and III are alike, and coincide with that prepared by Dr. Stephens from the case of Armstrong in Liverpool, whereas Curves II, IV, and V. approach more to the type described by Kinghorn and Yorke from the Luangwa Valley."

In a table showing the percentage of posterior-nuclear forms in the blood of rats inoculated with all the five strains it is seen that it varies from 3·3 to 34·1, with an average of 17·8.

"It is to be noted that in the human strain the percentage of posterior-nuclear forms varies greatly, although the method of enumeration is the same in each case. This presence of posterior-nuclear forms would have been accepted a few months ago as sufficient proof that the species dealt with was *T. rhodesiense.* Since then the posterior nuclear forms have been reported as occurring in *T. brucei* from Egypt, Uganda and Zululand. In a strain lately obtained by Theiler from the same spot in Zululand where this species was originally discovered in 1894, this percentage rose to the highest yet recorded."

Among the conclusions drawn is the following:—

Evidence is accumulating that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are identical.

(392) Bruce (D.), Harvey (D.), Hamerton (A. E.), Davey (J. B.), & Lady Bruce. **Trypanosome Diseases of Domestic Animals in Nyasaland. II.**—*Trypanosoma caprae* **(Kleine).**—*Proc. Roy. Soc.* 1913. Apr. 7. Series B. Vol. 86. No. B 587. pp. 278-284. With 1 plate.

This trypanosome belongs to a group which comprises *T. uniforme, T. vivax,* and *T. caprae.* They are all characterised by their extreme motility, clear cell contents, large round

terminal micronucleus, and by the fact that they are not pathogenic for the small laboratory animals. They all develop in the proboscis of flies and not in the alimentary canal. *T. caprae* is transmitted by *G. morsitans*, and the others by *G. palpalis*.

The description of *T. vivax* in the unstained state can, it is said, be equally applied to *T. caprae*.

In the preparation of stained smears the procedure adopted was fixation with osmic acid and staining with Giemsa.

From measurements of the trypanosome in the ox, sheep, goat, and waterbuck the following figures were arrived at:—

Species of Animals.	Number of Trypanosomes measured.	Average Length.	Maximum Length.	Minimum Length.
Ox	40	25·7	32·0	18·0
Goat	260	25·3	31·0	20·0
Sheep	180	25·6	32·0	21·0
Waterbuck	20	26·8	29·0	25·0

Total number of trypanosomes measured 500.

A curve constructed upon the measurements of these 500 individuals shows that the organism is monomorphic, the greatest number of trypanosomes (19 per cent.) measuring 25 microns in length.

Measured across its broadest part *T. caprae* measures on an average 3 microns (maximum 4·25, minimum 1·75).

T. caprae differs from *T. vivax* in that it is heavier built and of a more clumsy appearance. The posterior half is swollen, and its end bluntly angular or rounded.

The cell contents have a delicate alveolar structure and are free from granules or vacuoles.

The nucleus is oval and compact and lies about the middle of the body.

The micronucleus is large and rounded and as a rule lies close to the posterior extremity.

The undulating membrane is more developed and is thrown into bolder folds than in *T. vivax*.

There is a well-marked free flagellum which averages 6·5 microns in length.

It would appear from the inoculation of two oxen that the trypanosome is not responsible for serious disease in that animal, as trypanosomes appeared in the blood, in small numbers and for a short time, two months after inoculation, and then disappeared.

In the sheep and the goat the disease set up by this parasite runs a fairly fatal course. Of 36 goats 15 were infected by wild *G. morsitans* and died, the period of infection being from 53-59 days. Four that were inoculated with infective blood died, on an average, in 57 days. The remaining 17 were alive at the time of writing, after intervals of from 61 to 262 days.

Of four sheep inoculated, three died in from 36 to 221 days, the fourth surviving (245 days).

Monkeys, dogs, rats, and guinea-pigs failed to become infected.

The carrier of the trypanosome is *G. morsitans*, and in experiments made to ascertain with what trypanosomes the wild flies are naturally infected *T. caprae* was found in 61 per cent.

The development of the trypanosome in the fly is left for a further communication, but it is stated that it is restricted to the proboscis and runs a course of from 16 to 20 days.

With regard to the reservoir of the trypanosome, it has been found in 10·5 per cent. of 180 specimens of wild game.

(393) Macfie (J. W. Scott). **Trypanosomiasis of Domestic Animals in Northern Nigeria.**—*Ann. Trop. Med. & Parasit.* 1913. Mar. 31. Vol. 7. No. 1. pp. 1-26. With 3 plates.

During the greater part of 1912 the author was stationed in Ilorin, a province which is not considered unsuitable for horses save in one division, but within a period of eight months ten out of 15 horses in the possession of Europeans contracted trypanosomiasis and six died. In an attempt to introduce animal transport into the province ten donkeys were employed for the purpose between Ilorin town and Agugi, about 30 miles to the east. All were dead of trypanosomiasis within three months. At Zungeru in 1911 25 horses were under treatment, and of these 40 per cent. died or were destroyed. At Lokoja the disease is even more serious and the percentage of dead or incapacitated horses is placed at 73. It is a matter of impossibility to estimate the losses sustained by the natives.

Cattle, sheep, goats, and dogs contract trypanosomiasis, and diseased cattle are killed and sold for food. Ilorin is situated at the point where the main routes from Kano and Sokoto converge and the majority of the animals slaughtered for the market are affected with trypanosomiasis. Examination of 35 animals killed for the market showed that two were infected with *T. brucei*, 30 with *T. vivax*, two with *T. pecorum* or *nanum*, and in one case there was double infection.

In 1911 a list of identifications was published of 15 cases of trypanosomiasis in horses collected in Northern Nigeria. The author has now added a number of fresh cases, both in horses and in other domestic species, bringing the total number up to 86.

The following table gives an analysis of the results of the examinations:—

Host.	*T. brucei.*	*T. vivax.*	*T. nanum* or *pecorum.*	*T. theileri.*	Double infection.
Horse	14	18	8	—	3
Donkey	2	2	—	—	—
Cattle—					
Fulani	1	18	1	—	1
Dwarf	—	2	—	1	—
Sheep	1	8	1	—	—
Goat	—	4	—	—	—
Dog	—	1	—	—	—
Totals ...	18	53	10	1	4

As no systematic experimental inoculations could be carried out the parasites had to be identified by their morphological characters only.

The *T. brucei* referred to is, according to the author, in all probability the trypanosome for which STEPHENS and BLACKLOCK have suggested the name *T. ugandae*. In four cases (three horses and one donkey) of infection with *T. brucei* posterior nuclear forms were detected. In rats and guinea-pigs inoculated from these cases they appeared at certain stages of the disease in fairly large numbers. In a number of films the long forms were observed to have their posterior extremities blunted and almost rectangular. Great variations were observed from day to day in the relative percentages of the long and short forms, and a table is given showing the percentages of the different forms occurring in the blood of a horse, and of a rat and guinea-pig inoculated from it. The parasites are classed as "long forms with free flagellum" and "stumpy and intermediate forms" no measurements having apparently been taken.

It would appear that the disease in equidae is invariably fatal and two forms are clinically recognisable. Acute cases terminate fatally in from two to four weeks, but in chronic cases the disease runs a course of some months. It was noticed that all the cases in which posterior nuclear forms were observed were of the acute type.

T. vivax.—A trypanosome of this type was the one most commonly observed, for, as shown by the table, it was encountered in 56 of the animals examined (53 pure infections and three mixed). In view of the fact that it was found in the dog it was in all probability the *T. vivax* (Ziemann) and not the allied *T. cazalboui* as the latter is not pathogenic for the dog.

The disease caused by this parasite in horses would appear to be mild, since 14 out of 15 infected horses recovered, and in the fatal case the infection was complicated by a simultaneous infection with *T. brucei*. It is difficult to gauge the mortality of the infection in sheep and cattle as the animals in which the trypanosome was seen were slaughtered.

Short trypanosomes of the *nanum* or *pecorum* type were observed in 13 cases, of which ten were horses.

Trypanosomiasis is most common during the rainy season when the tsetse flies are most widely distributed over the country.

The province of Ilorin is peculiar in that whilst *G. palpalis* and and *G. tachinoides* are distributed all over it, tsetse flies of the the *morsitans* group—*G. submorsitans* and *G. longipalpis*—are restricted to the eastern division.

It is possible that in the native towns and in the European stations flies of the genus Stomoxys may play a part in the transmission of trypanosomiasis. One horse which had not been within two miles of any place known to be a fly district for five weeks previous to the onset of symptoms had been tormented by Stomoxys flies which were exceedingly common at the time. Both *S. nigra* and *S. calcitrans* have been captured at Ilorin.

Arsenic and perchloride of mercury have been administered both as curative and prophylactic agents but without success.

"No immunity follows an attack of trypanosomiasis, reinfections with the same or a different species of trypanosome being met with."

With regard to the dwarf cattle a paper by Foy is quoted as follows:—

"That a certain breed of cattle found in pagan districts possess a high degree of natural immunity in that they may harbour the trypanosome in the blood and yet keep in good condition and show no signs of the disease, nor do they die from the infection so long as their environments are favourable. These environments are a free life, with ample food, especially plenty of green grass. Confinement, poor feeding, and hard exercise, tend to make the disease manifest itself clinically.

"That such domesticated cattle may act as a reservoir of infection since the blood may prove infective at such times when clinical symptoms manifest themselves, although the trypanosome may not be found on making a microscopical examination."

From another part of the same paper it is quoted that all the trypanosomes met with were of the *T. brucei* type.

Details are given of two experiments carried out on these dwarf cattle, and the results appear to indicate that they, like horses, possess an immunity to *T. vivax*. The immunity however does not appear to be serviceable against *T. brucei*.

A tabular synopsis of the cases of trypanosomiasis in domesticated animals collected in Northern Nigeria is appended.

(394) Balfour (A.). **Animal Trypanosomiasis in the Lado (Western Mongalla) and Notes on Tsetse Fly Traps and on an Alleged Immune Breed of Cattle in Southern Kordofan.**—*Ann. Trop. Med. & Parasit.* 1913. Mar. 31. Vol. 7. No. 1. pp. 113-120. With 2 plates.

The author found trypanosomes in smears of blood taken from transport bulls working on the road running from Rejaf on the Nile to Aba on the Congo frontier. Measurements were taken of 140 specimens and from the results obtained it would appear that the parasite agrees very closely with *T. nanum* occurring in the Sudan. The maximum length of the trypanosome was 17 microns, the minimum 9·8, and the average length 12·6. The parasites varied in breadth from 1 to 2 microns. The infection in none of the three cases was heavy, but the author is informed that it is proving fatal to the cattle. A dog was inoculated with blood containing the trypanosome and parasites appeared in its blood fourteen days after inoculation and then disappeared, and were not found again. The dog remained in good condition. A number of rats which were inoculated died within 2 days from some unknown cause.

If the infection has been derived along the road in Western Mongalla the vector is in all probability *G. palpalis*, as *G. morsitans* is not found in this district, and the only other biting flies in evidence are *Haematopota*. It is, however, probable that the cattle were infected before going to Western Mongalla, and that some species of *Tabanus*, *Chrysops*, or *Stomoxys* is the active agent in transmitting this form of animal trypanosomiasis in the Southern Sudan.

One donkey was found to be infected with trypanosomiasis at Yei, and from an examination of the parasites contained in a single smear it would appear to be not unlikely that the organism was *T. congolense*.

In mules at Yei a trypanosome of the *brucei* or *gambiense* type was found, but gerbils inoculated with blood containing the parasite failed to become infected.

A hundred trypanosomes were drawn and measured and the following results obtained: Minimum length 10·5, Maximum 28, Average 17·6. The trypanosome appeared to be dimorphic, short stout forms without free flagellum and long slender forms with a free flagellum occurring. The short forms measured from 2·5 to 3 microns in breadth and the slender forms 1·2 to 2 microns. In the majority of the short forms there was an unstained area around the blepharoplast, and this was also seen in some of the slender forms.

The breed of small animals said to be immune to trypanosomiasis comes from Southern Kordofan, and it is the only breed that can live in the Koalib district, where *G. morsitans* abounds and conveys an infection which the author believes to be *T. brucei*.

(395) Duke (H. L.). **Some Observations on *Trypanosoma pecorum* (Bruce) and *T. uniforme* (Bruce).**—*Proc. Roy. Soc.* 1912. Oct. 11. Series B. Vol. 85. No. B 582. pp. 554-561.

Trypanosoma pecorum.

This trypanosome, according to Bruce and his collaborators, is the cause of a rapidly fatal disease in cattle and domestic animals generally. It appears to be widely distributed throughout Uganda, but nothing definite is known regarding its true carrier in nature. The disease is met with in places where tsetse are unknown and *G. palpalis* would appear from experiments to be at the most a facultative host. Nothing is known regarding a natural reservoir for this trypanosome.

The experiments recorded in the first portion of the paper were carried out with the object of ascertaining whether *T. pecorum* is pathogenic for antelope, and to test whether these animals can act as a reservoir for the trypanosome.

In an experiment, the details of which are tabulated, flies were fed on seven occasions on a bushbuck which had been infected with *T. pecorum* by inoculation from a monkey and from whose circulation trypanosomes disappeared after having been present for some days. These flies were then fed upon clean monkeys, and in only one instance was the result positive. Unfortunately in this instance there was a possibility than the monkey acquired the infection from a source other than the flies. On the occasion of one particular batch of flies having been fed upon the monkey none of the flies were found to contain flagellates, and it was thought that the experiment would of necessity be negative. This monkey was inoculated with blood from two out of three bushbuck shot, a goat being inoculated with mixed blood from all three at the same time. No trypanosomes were found in any of the smears made from these three buck, and the goat did not become infected,

nevertheless there is just the possibility that the monkey acquired the infection from one of the buck and not from the flies.

On dissecting the batch of flies which fed upon the monkey which became infected only one (out of 105) was found to contain flagellates.

A table is given showing the results obtained from the dissection of fifteen infected flies found in the batches used in the experiments with the bushbuck and in another series of experiments in which the infective feeds were obtained on a number of different animals. In the case of three of these flies observations were made upon the sucking stomach and in one only, the one from the batch which in all probability infected the monkey, there were found large numbers of flagellates, and it is suggested that may have a definite developmental significance in *T. pecorum*.

In only three of the flies were the salivary glands obtained, and all were negative. In this respect *T. pecorum* resembles *T. nanum*.

It may also be gathered from the table given that no proboscis infections were obtained before the 76th day of an experiment. Although four flies were obtained showing a good infection of the proboscis none of these were capable of infecting a monkey.

Possibly the flagellates present in the sucking stomach of the one fly were responsible for the infection of the monkey upon which it fed, or there may have been a proboscis infection which had disappeared before the fly was dissected.

It may be further gathered from the table that the bushbuck which was the source of the infection was still capable of infecting laboratory-bred *G. palpalis* three months after its original infection with *T. pecorum*.

Ten months after the original infection of the buck its blood was used in quantities of 2½ to 6 cc. for the inoculation of two monkeys and a calf. The calf, which received 3·5 cc., became infected.

The possibility of error is excluded from this experiment by the following facts:—

1. Since September, 1910, there had been no case of spontaneous infection with this trypanosome among laboratory cattle.

2. The cattle in the neighbourhood of the hill were apparently free from the disease.

3. A calf about the same age as the one used in the experiment had been under observation for some months and had never developed trypanosomes. The two animals had never been allowed to leave the hill top, and had always been stalled together.

The buck that was the source of the infection had not up to the time of writing shown any evidence of having been harmed by the infection. The calf which was successfully infected from it died in 51 days.

The long sojourn of the trypanosome in the bushbuck did not render it more suited to development in *G. palpalis*.

Trypanosoma uniforme.

This organism appears to be the most common antelope trypanosome in the Mpumu neighbourhood. According to Bruce it is responsible for a rapidly fatal disease in domestic ruminants, death occurring in laboratory infected goats in 29 days.

The present author's experience leads him to form a contrary opinion. During a period of 20 months only one animal has died of the infection.

A characteristic feature of *T. uniforme*, which is especially marked in goats, is the manner in which, after a few weeks, it totally disappears from the peripheral blood, as regards ordinary routine examinations, while the animal shows no symptoms whatever.

When this trypanosome occurs together with *T. vivax*, or possibly with *T. gambiense*, death not uncommonly occurs after rapid emaciation and some paralysis have been observed. This, however, is not constant.

Tabulated details are given of the course of the disease in four goats, four calves, one bushbuck, and one sheep. Only one of the calves died, apparently from the infection. The death took place 18 months after inoculation (calf already mentioned).

The strain employed was originally obtained from a bushbuck, while that employed by Bruce was obtained from oxen. This may possibly explain the difference of virulence.

Antelope as reservoir for T. uniforme.

In one out of a series of five experiments it was shown that a situtunga was capable of infecting *G. palpalis* with *T. uniforme* after a period of ten months, although the animal remained in excellent health.

(396) Duke (H. L.). **Further Investigations on the Rôle of Antelope as a Reservoir of** *T. gambiense*. — *Reports of the Sleeping Sickness Commission of the Royal Society.* 1913. No. 13. pp. 58-66.

In a previous paper (see this *Bulletin* Vol. 1. No. 2. Abst. No. 135. pp. 94-95) experiments were described dealing with the duration of infection of antelope with *T. gambiense*, and the following conclusions were arrived at: —

1. The antelope may remain capable of infecting *G. palpalis* with *T. gambiense* for a period of at least 22 months after their original infection.

2. There is some evidence to show that an antelope which has ceased to be infective for *T. gambiense* acquires some degree of immunity against re-infection.

In the present paper the history of these antelope during the period March 1912 to November 1912 is followed out.

Reedbuck 2357. The blood of this animal had last been proved infective eight months previously. It was fed upon by a number of flies which were infected and of which eighteen had positive salivary glands. After the lapse of an interval to allow the trypanosomes to make their appearance in the blood this animal was tested with clean laboratory-bred flies and by inoculation of its blood into susceptible animals. The flies after feeding were transferred to a clean monkey which they failed to infect. One monkey out of five inoculated became infected.

Reedbuck 2431. Last evidence of infection obtained fourteen months previously. On two occasions this animal was fed upon

by infected flies (four flies with positive salivary glands). Clean flies fed upon it infected two monkeys upon which they were afterwards fed. One monkey out of a batch of five inoculated became infected.

In a small number of experiments with white rats the serum of this animal was found to have no protective action.

Reedbuck 2359. This animal which had shown no sign of infection for eight months was tested in a manner similar to that adopted in the previous cases and was found to be susceptible to re-infection.

Bushbuck 2371. No attempt was made to re-infect this animal but its infectivity was tested with negative results (Control).

Waterbuck 2378. The tests of infectivity and attempts to re-infect were carried out as before, and the serum of the animal was tested for protective properties. It was found that the buck had acquired a degree of immunity which enabled it to resist re-infection by positive *palpalis*. Its serum was found to have no protective powers (rat experiments).

Bushbuck 2328. This animal was subjected to two attempts to re-infect it with positive *palpalis* and in each instance without result. It further resisted inoculation with the blood of an infected monkey. Its serum was without protective properties.

(397) Canada: Department of Agriculture. **Report of the Veterinary Director-General and Live Stock Commissioner, J. G. Rutherford, C.M.G., for the Year ending March 31, 1912.** —480 pp. With 29 plates. 1912. Ottawa. [Sessional paper No. 15b—1913.]

Dourine.

Dourine still exists to a limited extent in Southern Alberta where its presence was first discovered in 1904. The figures show that the number of horses slaughtered during the period under review (18) is far below that slaughtered in any previous year, and attention is drawn to the fact that in five instances the infection was traced to a stallion imported direct from Iowa.

(398) Robertson (Muriel). **Notes on the Life-history of *Trypanosoma gambiense*, with a Brief Reference to the Cycles of *Trypanosoma nanum* and *Trypanosoma pecorum* in *Glossina palpalis*.—*Reports of the Sleeping Sickness Commission of the Royal Society*.** 1913. No. 13. pp. 119-142. With 6 plates.

An abstract of the portion of this paper dealing with *T. gambiense* has already appeared in this *Bulletin* (1913. Vol. 1. No. 3. pp. 154-155.); the short section dealing with *T. nanum* and *T. pecorum* in *G. palpalis* may therefore be considered.

The development of *T. nanum* in *G. palpalis* has many features which closely resemble the conditions observed in connection with *T. gambiense*. The infection starts in the hind gut and by the 10th day there is an extensive invasion of the hind and middle gut. The method of division corresponds almost exactly with that observed in *T. gambiense*. Slender forms appear at the

10th to 14th days, and the proventriculus is invaded by the 20th day. The trypanosomes present in the proventriculus are not so uniform as in *T. gambiense* and the changes observed in the nucleus of the latter trypanosome do not occur in *T. nanum*. By the 25th day the parasites have invaded the proboscis and there assume the crithidial form.

The gut forms do not attach themselves to the gut wall, and crithidial forms are never observed in the gut cycle. The salivary glands are never invaded by this trypanosome.

The cycle of development of *T. pecorum* in *G. palpalis* is very slow, and transmission difficult to effect. Duke's experiments indicate that the fly is at the most a facultative host of the trypanosome. The course of development resembles that of *T. gambiense* and *T. nanum*, but the flagellates are larger and more massive than in the two organisms just mentioned. The slender forms are extraordinarily long and the nuclear changes seen in *T. gambiense* occur in this parasite. The first invasion of the proventriculus was observed on the 45th day of the cycle, and no proboscis infection was found before the 76th day. The proboscis infections were generally slight. The salivary glands never became infected.

(399) Duke (H. L.). **Some Attempts to transmit *Trypanosoma gambiense* by Wild Stomoxys; with a Note on the Intestinal Fauna of these Flies.**—*Reports of the Sleeping Sickness Commission of the Royal Society.* 1913. No. 13. pp. 89-93. With 36 text-figures.

The wild flies used in these experiments were *S. nigra* and *calcitrans*. These flies were fed upon infected monkeys and then placed upon clean monkeys. In no instance did infection result. Although no flagellates having crithidial or trypanosome structure were found in any one of the flies dissected, a flagellate showing the structure of Bodo and a crescent shaped body were seen.

The latter appear to be non-motile and are often present in great numbers in the gut of the flies and the possibility is suggested that they may represent some stage in the life history of some sarcosporidian parasite.

(400) Duke (H. L.). **Notes on *Trypanosoma gambiense* and *Glossina palpalis*.**—*Reports of the Sleeping Sickness Commission of the Royal Society.* 1913. No. 13. pp. 13-21.

(A) *Observations on the Physiology of* T. gambiense *in* G. palpalis.

These experiments were undertaken with the object of ascertaining whether the nature of the blood ingested by flies during the earlier days of experiment had any effect upon the development of *T. gambiense* in them.

A series of paired boxes was taken and fed for the same period upon a monkey whose blood contained *T. gambiense*. Throughout the experiment both series received parallel treatment.

The following is an example of a pair of these experiments each of which served as a control to the other.

Experiment 358.

Date.	Day of Experiment.	Procedure.	Result.	Remarks.
1911.				
Sept. 3	1	Fed on monkey 199.		
„ 4	2	Starved.		
„ 5–25 ...	3–23	Fed on bushbuck 123.	—	6 positive flies dissected. Trypanosomes in gut only.
„ 26 ...	24	Starved... ...		1 positive fly died. Flagellates in gut and thoracic gut only.
„ 27–Oct. 7	25–35	Fed on monkey 390.	+	76 flies alive on 30th day.

At the final dissection six more positive flies were found, four of which showed virulent salivary infection. The number of infected flies was 11·5 per cent. of the total.

Experiment 357 (control to 358).

Date.	Day of Experiment.	Procedure.	Result.	Remarks.
1911.				
Sept. 2–3 ...	1	Fed on monkey 199.		
„ 4... ...	2	Starved.		
„ 5–25 ...	3–23	Fed on fowl 364		1 positive gut only.
„ 26 ...	24	Starved.		
„ 27–Oct. 6	25–34	Fed on monkey 391.	+	44 flies alive on 30th day.

At the final dissection one positive fly was found with numerous flagellates in the salivary glands. The number of infected flies was 2·6 per cent. of the total.

A summary of the results obtained in the series of experiments is tabulated as follows:—

Experiment Number.	Percentage of positive flies with goat or buck blood.	Percentage of positive flies with cock or monkey blood.
357 & 358	11·5	2·6
567 & 568	3·5	0
581 & 582	20·6	3·6
617 & 618	3·7	0
127 & 128	0	3·3

The experiments summarised above represent all the work done in this connection up to the time of writing and have not been especially selected.

The results obtained are in marked contrast to those recorded by KLEINE and FISCHER who, using a far greater number of flies, obtained approximately 10 per cent. of infected flies when monkeys were used throughout the experiment and 2·4 per cent. when ruminants were used.

The possibility of the flies having picked up flagellates other than *T. gambiense* is practically excluded by the following control experiments.

Bushbuck 123 which had been infected with *T. uniforme*, a trypanosome readily transmitted by *G. palpalis* and confined exclusively to the proboscis, was tested on three separate occasions with a number of flies of which 180 were dissected with negative results. On two occasions its blood was used for the inoculation of clean monkeys again with negative results.

One of the sheep used in the experiments had never been infected at the laboratory and during a period of three months its blood was examined on 57 occasions with negative results. The other sheep used had been infected with *T. uniforme*, but although its blood had been examined from time to time for nearly a year no evidence of infection had been obtained.

KLEINE and FISCHER conclude that monkeys and man are far more important than ruminants as a reservoir for sleeping sickness, but the whole of the work done at Mpumu would point to the opposite conclusion.

(B) *To study the effect of different kinds of blood on the longevity of laboratory-bred* G. palpalis.

A table is given showing the details of ten experiments in which batches of flies numbering from 17 to 75 were fed for periods of two to four days upon different species of animals and then starved, the number of deaths in each batch being recorded daily. It is pointed out that the weak point in the experiment lies in the fact that it was impossible to obtain a number of flies of exactly the same age, and also to ensure satisfactory feeding of all the flies.

(C) *To prove that* G. palpalis *infective for* T. gambiense *is capable of infecting monkeys on three consecutive days.*

A batch of flies which had been shown to be infective was fed upon three monkeys on three consecutive days. Each of the monkeys became infected, and on dissecting the batch of flies only one was found to be infected, numerous flagellates being present in the gut and salivary glands. This fly must have infected all three monkeys.

(D) In a single experiment it was shown that the feeding of a large number of negative *Glossina palpalis* on a monkey does not interfere with the subsequent infection of the monkey with positive flies.

(401) Roubaud (E.). **Relations Bio-géographiques des Glossines et des Trypanosomes.** [The Bio-geographical Relationship between Glossina and Trypanosomes.] — *Bull. Soc. Path. Exot.* 1913. Jan. Vol. 6. No. 1. pp. 28-34.

Having frequently noticed the inconstancy and infrequence of salivary infection in flies, the author formed the opinion that local climatic conditions might influence Glossina in such a way as to render them unfavourable for the evolution of the cycle of trypanosome development, and suggested that strains of flies differing from each other with regard to the development of trypanosomes in their salivary apparatus might make their appearance. In 1910 an experiment was made with *T. cazalboui* which justified the conclusion arrived at. Later, in conjunction with Bouet, the observations were confirmed and the "partial disinfection of the Glossina under the physical conditions of captivity" was established.

Experiments with Glossina soon showed that if a batch of flies be given infective feeds only a proportion acquire salivary infection, and that if the number of feeds be increased the number of flies showing salivary infection is also increased, but that there are always some that do not show this infection. The author states that for the acquisition of this infection the flies may have to be placed in certain physiological conditions which he calls "receptivity." This applies only to the salivary infection and may be independent of infection of the alimentary canal. It may be temporary and disappear at the end of a certain time.

Under natural circumstances it has been shown that Glossina of different species frequenting the same district are not infected in the same proportions, nor in the same manner. In Lower Dahomey *T. cazalboui* predominates in *G. longipalpis* and *palpalis*, *T. dimorphon* in *G. longipalpis* and *tachinoides*, *T. pecaudi* in *G. longipalpis*. In Upper Dahomey, *T. pecaudi* predominates in *G. morsitans*, and in Casamance *T. dimorphon* in *G. morsitans*.

In view of the fact that wild domesticated animals exist in these areas under the same conditions, the author thinks that the differences observed are due to bio-geographical differences to which the flies are subjected. The author's own observations regarding the variation of receptivity exhibited by *G. palpalis* towards *T. cazalboui* support this view.

At Brazzaville in the Middle Congo 4·8 per cent. of *palpalis* became infected in his experiments (41 flies given one infective feed). At Agouagon in Middle Dahomey the average percentage was 40, but it varied from 10, when there was a single infective feed, to 70, where several infective feeds were given. Repeated infective feeds failed to infect a single fly out of 60 at Kolda in Upper Casamance.

Examination of naturally infected flies confirmed these differences. In Middle Dahomey one fly in 30 was found to have a proboscis infection, while in Upper Casamance 560 flies were examined and only one with proboscis infection found. Souma was of fairly frequent occurrence in the area, but it must have been transmitted by some other species of Glossina.

Comparison is made between the results obtained in the experiments regarding the transmission of *T. gambiense*, and those obtained by BRUCE and KLEINE, the latter authors finding as many as 5 per cent. of flies infected in this way.

In Dahomey where the disease does not exist not one fly in 1,200 was found to be receptive.

Mention is also made of KINGHORN and YORKE's results with *G. morsitans* and *T. rhodesiense*, in their experiments made in the Luangwa Valley and on the plateau.

The failures of KLEINE and FISCHER on the Victoria Lake, and the successes of TAUTE on Lake Tanganyika are also contrasted.

The conclusion drawn by the author is that the receptivity of a given species of Glossina for a certain virus is not uniform throughout the area of distribution of the fly, and that receptive, refractory, and indifferent strains occur in nature.

The author thinks that it is incorrect to state that climatic influences exercise an effect upon the development of the virus in the flies, at any rate directly. Bio-geographical factors produce modifications in the saliva which are favourable or otherwise to the development of the trypanosomes. This is shown by the fact that in a given area certain species are receptive and others are not. All other factors being the same, it must be the receptivity of the flies that varies.

If then the receptivity of the flies varies it follows that disease caused by trypanosomes transmitted by them will only be endemic in districts where receptive flies exist. These districts, however, are not constant owing to migrations of the flies. This possibly explains the occurrence of sporadic cases of trypanosomiasis in districts where flies are numerous, but where trypanosomiasis is not of common occurrence. In such areas cases of disease often tend to be of a mild type.

The author has frequently observed this in cattle and in horses in Gambia and Casamance. It is conceivable that geographical factors may exert an influence upon infected Glossina passing from an endemic zone to an indifferent one, in the direction of attenuating the virus, and even leading to absolute disinfection of the fly. Changes of this kind have been observed in the laboratory.

To sum up, the receptivity of the different species of flies in a district must be ascertained before any conclusion as to the endemicity of a disease can be arrived at, and the presence of a large number of flies must not be taken as evidence that such an area is dangerous either to man or animals. The introduction of a virus into a district in which flies are present is not of necessity dangerous, and the author believes that the chances of the extension of a virus outside areas of normal receptivity are very slender.

Any rational campaign against the flies must therefore be directed against the areas of receptivity, and measures of deforestation should be preceded by experimental investigation of the receptivity of the flies in the area, or at least by an examination of their natural transmitting power.

(402) Robertson (Muriel). **Notes on the Polymorphism of *Trypanosoma gambiense* in the Blood and its Relation to the Exogenous Cycle in *Glossina palpalis*.—*Reports of the Sleeping Sickness Commission of the Royal Society.*** 1913. No. 13. pp. 94-110. With 26 charts.

This paper deals with the fluctuation of the number of trypanosomes present in the blood of an infected animal, and with the question of polymorphism.

Attention is drawn to the evidence supporting the view that multiplication of the parasites occurs in the circulating blood and that it is improbable that invasion of the cells of the host for the purpose of multiplication occurs. The factors governing the number of trypanosomes present in the blood at any given time are discussed at some length.

The preparations used in the investigations of the polymorphism of the parasite were fixed first with osmic acid vapour and then with alcohol, being afterwards stained with Giemsa. The parasites were measured, after having been drawn with a camera under a magnification of 2,000, by Bruce's method.

There is no sharp division of trypanosomes into types, the forms merging into each other. It is believed that the short forms represent the "adult blood type," so called because it is the most stable form. These become elongated and more slender and, passing through the intermediate stage, reach the long slender form. These long forms divide to produce the short forms again.

The question whether any special conditions of the parasite or of the infection are essential for the production of positive flies is considered and the view is held that an average number of positive flies is obtained when there has been a drop in the number of trypanosomes in the blood and those present are mostly of the short type.

(403) Ogawa (M.). **Quelques Observations sur le Dimorphisme de *Trypanosoma pecaudi*.** [The Dimorphism of *T. pecaudi*.]—*Centralbl. f. Bakt.* 1. Abt., Orig. 1913. Mar. 15. Vol. 68. No. 3-4. pp. 332-334. With 3 text-figures.

During the course of an infection with *T. pecaudi*, the cause of Baleri, two forms of the parasite are observed, one long and slender and the other short and stumpy. In his original investigations Pecaud was inclined to think that he was dealing with a double infection, but was never able to furnish proof of this.

The author has made observations with the object of ascertaining whether any connection exists between the course of the disease and the occurrence of the two forms of parasite in the blood, and for this purpose has adopted the biometric method. Long forms and dividing forms make their appearance in the blood some days before the short stumpy forms.

In animals like the guinea-pig in which the course of the disease is (as a rule) sufficiently long, the short forms occur in large numbers during the middle period of infection, while during the last stage the long forms again predominate.

In animals like the mouse, in which the course of the disease is rapid, the long forms outnumber all other forms during the whole period of infection.

Measurements were taken of trypanosomes occurring in the blood of two guinea-pigs and a chart constructed upon the results obtained.

The first guinea-pig survived until the 16th day after subcutaneous inoculation with blood containing a large number of parasites, and the second, inoculated in a similar manner, died on the 7th day. The measurements were made on smears fixed while moist with osmic acid and stained with Giemsa. In the case of the first guinea pig 1,000 trypanosomes were measured, and in the case of the second 200.

The long forms measured from 24 to 34 microns in length and from 1 to 1·5 in width. The short stumpy forms without free flagellum varied from 12 to 20 microns in length and from 2·5 to 4 in width. Intermediate forms provided with a short free flagellum measured 21 to 23 microns in length and 1·5 to 2 in width.

From the chart given it may be gathered that in the first guinea-pig there were trypanosomes varying in length from less than 12 microns up to 33 microns, whereas in the second guinea-pig the shortest trypanosome encountered measured 18·5 microns in length and the longest 32 microns. In both animals the trypanosomes measuring about 25 microns were the most numerous, but in the first the percentage of these was only just over 6, while in the second the percentage was 11.

The author's examinations of smears containing *T. pecaudi* enable him to support the statements made by Wenyon, Laveran, and Nattan-Larrier that posterior nuclear forms of *T. pecaudi* occur. In rare instances the nucleus was actually posterior to the blepharoplast.

The author has been unable to find any indication of sexual forms.

(404) Duke (H. L.). **A Trypanosome from British East Africa Showing Posterior-Nuclear Forms. (With a Note on its Developmental Stages in *G. palpalis*.** By Muriel Robertson.)—*Reports of the Sleeping Sickness Commission of the Royal Society*. 1913. No. 13. pp. 67-89. With text-figures.

This organism, which was obtained from Montgomery, was originally derived from a donkey which was supposed to have contracted the disease at a place a day's march on the road from Ngobotok to Baringo. According to P. Ross tsetses are known at three places on that road and the species found are *G. pallidipes*, *G. longipennis* and *G. brevipalpis*. The disease is described by Crowther as lasting four days.

In the living state the trypanosome executes very active and ceaseless wriggling movements with the anterior portion of the body while the posterior portion may remain almost motionless. There is very little translation, but when this does occur the movement is of a steady gliding nature.

In fixed and stained preparations a varying number of granules may be seen in the body. These may be anterior or posterior to the nucleus, or scattered throughout the body. They are most frequently observed anterior to the nucleus.

The kinetonucleus is as a rule not quite terminal, and on rare occasions it may be seen unusually far forward.

The trophonucleus is in a very considerable number of cases situated in the posterior portion of the body, and often quite close to the posterior end. This arrangement is more frequently observed in the short and intermediate forms. The proportion of posterior-nuclear forms varies in different species of hosts. They are relatively common in the dog and rat, and rare in monkeys. Occasionally they may number thirty per cent. of the parasites present.

The undulating membrane is well marked.

In the long forms there is a long free portion of the flagellum. The stumpy forms usually have a short free portion, but in some the protoplasm extends to the tip. In certain specimens a clear vacuole measuring as much as three microns in diameter is seen just anterior to the kinetonucleus.

On one occasion an individual was seen in the circulation of a dog which suggested a process of multiple division.

In a certain proportion of specimens a staining line may be seen passing from the kinetonucleus to the trophonucleus, and thence to the anterior end of the body. The anterior portion of this line may sometimes shew bead-like thickenings. In wet preparations stained by Heidenhain's method the line is also visible, but the process of differentiation may be carried too far to allow either the line or the granules to be seen.

When the trypanosome was first sent to Mpumu Montgomery mentioned that it was supposed to be *T. pecaudi*. This is of interest in view of Wenyon's statement regarding the occurrence of posterior nuclear forms in *T. pecaudi* (see this *Bulletin*, 1913, Vol. 1, No. 2, Abst. No. 140, p. 99).

The trypanosome varies from 12 to 38 microns in length, and is markedly dimorphic.

The value of the biometric method is discussed at some length and some comparisons instituted, the conclusion being drawn that at present the method is unsatisfactory for the diagnosis of species.

Tables are given shewing the results of infection with this trypanosome of a number of different species of animals, and it is seen that broadly speaking the reactions obtained agree with those obtained by Laveran, Cazalbou, and Bouffard with *T. pecaudi*. Guinea-pigs, however, appear to react in an exceptional manner. Five were inoculated from an infected dog and in one only were trypanosomes found. The period of incubation was 44 days. Bouffard's observations furnish a parallel to this, for he found that with *T. pecaudi* the period of incubation was never less than 20 days and in one instance was 53 days. An attempt to infect guinea-pigs with positive *G. palpalis* failed.

Although the general reactions of the trypanosome most closely agree with those of *T. pecaudi* (Bouffard's strain) the possibility

is considered that the parasite may be either a mild strain of *T. brucei* or *T. rhodesiense*. The lack of objective symptoms in sheep and goats appears to exclude *T. rhodesiense*, as does the general scale of virulence.

Experiments made with human serum shewed that the trypanosome is susceptible to the action of this serum either as a curative or as a protective, but the former action would appear to be weak. Though the protective action is well marked complete protection was not observed.

The trypanosome can be transmitted by *G. palpalis* and it would appear that in infective flies the trypanosomes occur in the gut and salivary glands, but not in the proboscis.

The question of the diagnosis of species of trypanosomes by a study of the distribution of the parasites in the intermediate host is considered at some length. The trypanosome described in this paper belongs to the gambiense-rhodesiense group in that it develops in the gut and the salivary glands and not in the proboscis of *G. palpalis*. The *T. pecaudi*, to which it bears some resemblance in other respects, belongs to the group of trypanosomes which develop in the gut and the proboscis of the transmitting flies—"infection totale" of Roubaud.

Note on the Life Cycle of the Trypanosome in the Alimentary Tract of Glossina palpalis.

The development of this trypanosome in *G. palpalis* is in the main very like that of *T. gambiense*, but there are certain differences which enable the two to be distinguished.

The earliest evidence of development was seen in a fly on the third to fourth day after infection. The trypanosomes had increased somewhat in size and bulk and were extraordinarily uniform in type. Division was in active progress and the parasites had spread through the mid and hind gut. The details of division are identical with those of *T. gambiense* (see this *Bulletin* 1913, Vol. 1, No. 3, Abst. No. 285, pp. 154-155). It is stated that the details of division of the four trypanosomes *T. nanum*, *T. pecorum*, *T. gambiense*, and the organism at present under consideration correspond so closely that one set of figures would correctly illustrate the process in any one of them.

By the seventh day the trypanosomes in the mid and hind gut begin to lengthen. Certain very characteristic types are found about this period; they are distinguished by the body being drawn out rather sharply and stiffly posteriorly, and in the living state they have a peculiar stiff vibratile motion. About the fourteenth day long slender trypanosomes appear in the proventriculus where they form the predominant type.

At this stage there is often a period during which no further development takes place, but after this interval the slender proventriculus forms may be found in the hypopharynx and then in the salivary glands. These forms are apparently not capable of infecting and the development of typical salivary forms appears to be absolutely necessary.

The slender forms attach themselves in or near the duct. They then become broader in shape, divide, and migrate backwards along the gland where they re-attach themselves. They

assume the crithidial state and become quite rounded in shape. Multiplication goes on and this as a rule appears to be binary. The gland becomes crammed with parasites and the crithidial forms gradually develop into trypanosomes. This takes place about the 40th day. No evidence of conjugation has been observed in any phase of the cycle.

The features in which the cycle differs from that of *T. gambiense* are:—

1. The form under discussion is characterised by a rapid and numerous early development in the mid and hind gut, the early infections showing far greater numbers of parasites than *T. gambiense*.

The type of trypanosome present at this period shows a striking uniformity in shape and size.

2. There is in the trypanosome here described a marked pause between the first appearance of the trypanosomes in the proventriculus and their invasion of the salivary glands, which may amount to as much as 15 to 20 days.

3. The gland infections show far higher numbers than in the cycle of *T. gambiense*, but the development in the glands in the strain under observation occupied a considerably longer period than is the case with the sleeping sickness organism.

(405) Blacklock (B.). **A Study of the Posterior Nuclear Forms of *Trypanosoma rhodesiense* (Stephens and Fantham) in Rats.**—*Ann. Trop. Med. & Parasit.* 1913. Mar. 31. Vol. 7. No. 1. pp. 101-112. With 1 text-figure.

The experiments detailed in this paper were carried out with the object of determining the time of appearance of posterior nuclear forms in the peripheral circulation of rats and the numerical relationship which these forms bear to other forms of trypanosomes present in the blood from day to day.

A series of four groups of rats were inoculated intraperitoneally with measured doses of trypanosomes and the average duration of the period of incubation and of the course of the disease noted in each case. It was found that no definite variation of these periods could be discovered in the different groups, in spite of the fact that the dose in one group was eight times as large as that given in another group (8 million and 1 million). The average period of incubation was 4·5 days, and the average duration of the disease 15·5 days. Daily examinations were made of the blood, and in each film 200 trypanosomes were counted. In counting these every type of parasite whether dividing or not was included, and the number of trypanosomes showing posterior nuclei was noted. The posterior nuclear forms were classified according to the position occupied by the nucleus, and three groups were made: A, those in which the nucleus was only just posterior to the middle point of the body; B, those in which the nucleus was placed midway between the mid-point of the body and the posterior end; C, those in which the nucleus was quite close to the blepharoplast.

The first trypanosomes which appeared in the peripheral blood were of the long and slender, or intermediate types. Subsequently

short forms made their appearance, and after a few days posterior nuclear forms. The A form appeared first, then the B form, and lastly the C form. Occasionally A and B forms appeared together, and also A and C forms made a simultaneous appearance, but in no case was the C form alone observed as the first type of posterior nuclear form to occur in the blood.

From the counts made it appeared that the posterior nucleated forms increased in numbers from the time of their appearance, both actually and relatively to the other forms of the trypanosomes.

The constancy of the results obtained by counting 200 parasites in each film was controlled by counts made of a thousand trypanosomes in each daily film from two of the rats in each group. The results obtained confirmed those obtained in the preliminary counts.

In several rats from which films were made after death it was observed that the proportion of posterior nuclear forms to other forms increased considerably.

The various views that have been expressed to account for posterior nuclear forms are considered at some length. These explanations are:

(a) The occurrence of such forms as a constant constituent of certain strains.

(b) A mixed infection.

(c) Certain unexplained influences in the blood environment affecting the parasites.

(d) The transmitting agent.

The arguments against the first of these explanations are weaker than those against the others.

The occurrence of posterior nuclear forms in animal trypanosomes is referred to, and it is said that "the fact that such forms are found in animal strains does not diminish their claim to attention, since animals are known to be infected with human trypanosomes. The argument, that if so many animal strains are capable of infecting human beings there would be a great amount of human trypanosomiasis in regions where it does not at present abound, is not conclusive. It has to be definitely proved that there is actually no human trypanosomiasis in such animal-infected regions. The experience in Nyasaland, and in Rhodesia, Northern and Southern, renders it necessary to be cautious in considering a region free from sleeping sickness."

(406) Stephens (J. W. W.) & Fantham (H. B.). **Further Measurements of *Trypanosoma rhodesiense* and *T. gambiense*.** —*Ann. Trop. Med. & Parasit.* 1913. Mar. 31. Vol. 7. No. 1. pp. 27-39.

This paper contains detailed measurements of 1,000 trypanosomes (non-dividing) taken from a single rat infected with *T. rhodesiense* and from another infected with *T. gambiense*, the measurements being taken, 100 daily, on consecutive days.

In each case the whole of the figures are given, and from them various tables are constructed.

In Table III. the trypanosomes are classified in Bruce's three groups (a) 13 to 21 microns; (b) 22 to 24 microns; (c) 25 microns and upwards, and it clearly shows that there is a great daily variation in the proportions of the different groups present in the blood, thus rendering a series of measurements made from preparations made from an animal taken at random liable to considerable inaccuracy.

The whole of the steps are repeated with *T. gambiense*; the measurements of 1,000 trypanosomes, measured 100 daily, from a single infected rat, and the tables of averages and groups as before.

From the table showing the group divisions of the trypanosomes it is seen that the daily variation of the percentage of the different forms present is even greater in the case of *T. gambiense* than in *T. rhodesiense*.

The measurements of both trypanosomes are represented graphically.

It is seen from the results obtained that the average, maximum, and minimum measurements of *T. gambiense* are a little greater than those of *T. rhodesiense* and that comparing the trypanosomes according to Bruce's groups the most marked differences lie in the percentage of short and long forms, the intermediate forms being present in about the same percentage in each case. Short forms predominate in *T. rhodesiense* to the extent of about 7 per cent. and long forms predominate in *T. gambiense* to about the same extent.

The authors state that there is a fair correspondence between their own curves and those constructed by Bruce, but none between their own and those constructed by Kinghorn and Yorke. They believe that these differences must be due to differences of method.

The authors have constructed three curves in all (1) based on 1,000 trypanosomes from various animals, but including 600 rat trypanosomes; (2) based on 600 trypanosomes from rats alone, where samples of 20 were taken on a number of days from different rats; (3) 1,000 trypanosomes taken 100 daily from one rat for ten days.

The three curves have this in common, that the peak of each is at 26 microns. Further, there is close agreement between curves (2) and (3).

The possibility is noted that trypanosomes taken direct from the natural vertebrate host or from the fly may have characters different from those that have been maintained in laboratory animals.

Conclusion:—

"We must admit that we had hoped to be able to distinguish between the two species, *T. rhodesiense* and *T. gambiense*, by measuring one thousand specimens of each organism. Though these biometric results are not sufficiently conclusive, we think that it is generally admitted that the two species are distinct.

Microscopically, the two trypanosomes are indistinguishable except by the posterior nuclear character of *T. rhodesiense*. We believe that a curve only expresses graphically what the eye can

appreciate under the microscope, and that if two trypanosomes cannot be distinguished microscopically, we shall not be able to do so by measuring them. However, provided that further experience enables observers to agree as to the best procedure, it is no doubt a great advantage to have a correct graphical expression for what is otherwise only an impression, although it may be a quite accurate one. Further, these measurements should not be regarded as useless, as they will undoubtedly form the basis (provided all the protocols are given) for a critical statistical investigation in the future."

In making the measurements the authors adopted their plan of projecting the trypanosomes directly on to a screen and tracing them, using the 'tangent line' method for the purpose of measurement.

(407) Favero (F.). **Contribution a l'Étude de la Differentiation des Trypanosomes.** [The Differentiation of Trypanosomes.]—*Recueil de Méd Vét.* 1913. Jan. 15. Vol. 90. No. 1. pp. 10-13.

The author's investigations were carried out in order to test the conclusions arrived at by Levaditi and Mutermilch.

According to these authors, leucocytes attack trypanosomes (alive or dead) only when the trypanosome used in the experiments in vitro is of the species which has been used for the infection of an animal, the serum of which has sensitised the leucocytes.

Favero's experiments had absolutely negative results in every instance, although the technique advised by Levaditi and Mutermilch was closely followed. Modifications of the technique did not lead to any better results.

Laveran and Thiroux, who have also tested the method, find that it has only a relative value, and that it is less accurate than the method of cross-immunity.

(408) Stephens (J. W. W.) & Blacklock (B.). **On the Non-identity of *Trypanosoma brucei*, Plimmer and Bradford, 1899, with the Trypanosome of the Same Name from the Uganda Ox.**—*Proc. Roy. Soc.* 1913. Mar. 6. Series B. Vol. 86. No. B 586. pp. 187-191.

In the introduction to this paper the authors briefly summarise the statements that have been published regarding the morphology of *T. brucei*.

Bruce states that the trypanosomes vary a good deal in shape and size, and that they appear to vary a little in form in different species of animals. One or two of his figures may possibly represent stumpy forms.

The description given by Kanthack, Durham, and Blandford suggests dimorphism, but no mention is made of forms without free flagellum. No slides were available for examination, but from the examination of a large series of photographs (Durham) it was found that, save for one or two doubtful stumpy forms, the parasite was markedly uniform in shape and size.

Plimmer and Bradford describe four forms, but neither their description nor figures suggest that stumpy forms were observed.

Bruce and others state that after comparison of old Zululand preparations with preparations from the Uganda strain they consider them to be identical.

In another paper Bruce states that *T. brucei* (Uganda strain) has 26 per cent. of non-flagellate forms.

Laveran classes *T. brucei* among the monomorphic trypanosomes.

The present authors have made a detailed examination of the two strains in a series of slides throughout the entire period of infection in various animals, viz., rats, guinea-pigs and rabbits.

The Zululand strain of trypanosome was derived from "a dog infected by the disease on the voyage from Africa, and brought to England in November, 1896, by Dr. Waghorn."

The Uganda strain was obtained originally from the Uganda ox in 1909. In 1912 this strain was lost for a short time, but was returned by Mesnil, who received it from Liverpool.

The authors state that they have established the following facts:—

(*a*) The Zululand strain is typically monomorphic, and the trypanosomes possess a long free flagellum. Short forms resembling true stumpy forms are to be found, but prolonged searching is necessary to discover them.

(*b*) Laveran's strain of *T. brucei* is monomorphic. Examination of old Zululand slides lent by Nuttall, Skinner, and Plimmer show that the strain is monomorphic, although prolonged search reveals short forms, but these have not the somewhat indefinable characteristic appearance of true stumpy forms.

(*c*) The Uganda strain is typically dimorphic, true stumpy forms being easily found and occasionally in abundance.

The Uganda strain was kept by Mesnil in mice for nearly a year, and very few stumpy forms were to be found, but they immediately reappeared on the trypanosome being passed into guinea-pigs.

The authors suggest three explanations of the facts:—

1. That the strain that has been designated *T. brucei*, Zululand, is not this strain at all, but some other trypanosome inoculated erroneously during the course of inoculations extending over years. They do not think this view is tenable.

2. That Bruce may have sent to England a strain other than the one with which he was working in Zululand. This is thought to be all the more likely as Bruce was able to infect dogs from wild game, and there was no suspicion at the time of the multiplicity of animal trypanosomes in Africa.

3. That the strain originally sent to England was dimorphic, but that it has now become monomorphic. This may have come about in two ways:—

a. The strain was originally a mixture, the stumpy form having died out. They regard this view as not impossible, but cannot at present prove or disprove it.

b. The strain was originally dimorphic, but not a mixture, and that it has become monomorphic. Of such a change there

is not much evidence at present, but it has been noted by MESNIL, and confirmed by the authors, that the Uganda strain kept in mice for a year was almost, but not entirely monomorphic, but that in guinea-pigs it at once showed its normal characters.

The authors suggest that the Uganda trypanosome should be renamed *T. ugandae*.

(409) LAVERAN (A.) & ROUDSKY (D.). **Essais d'Immunisation contre les Trypanosomes pathogènes. — Trypanotoxines.** [Attempts to Immunise against the Pathogenic Trypanosomes.—Trypanotoxins.] — *Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 176-181.

This paper contains the results of some experiments in which the technique devised by BRAUN and TEICHMANN was followed save that the materials were prepared with aseptic precautions instead of sterilisation being effected by means of toluene. This was done because it was found that accidents occurred if every trace of toluene was not removed.

The material used for the immunisation was a powder composed of dry trypanosomes (*brucei*) which was suspended in salt solution. Each mouse received five doses of 2 cg. at intervals of five days, the inoculations being intraperitoneal.

In the first series of experiments an attempt was made to vaccinate eight mice, but each succumbed to a test inoculation with virus.

Two attempts to vaccinate two rabbits by a similar method also failed. The dose of dried trypanosomes given in these cases at each inoculation was 5 cg.

In the third batch of experiments serum was obtained from a rabbit which had received four intraperitoneal inoculations of 5 cg. of dried *T. brucei*. Two mice were given 1 cc. each of this serum by intraperitoneal injection, and on the following days were inoculated with blood containing *T. brucei* in very small numbers. Both of these mice died.

A third mouse was tested after the administration of the serum by inoculating it with *T. congolense*. This mouse became infected but apparently recovered. A control mouse inoculated with *T. congolense* became infected and was still infected at the time of writing.

In all the experiments control animals were inoculated with the test virus.

A second rabbit which received seven doses of powdered dry *T. brucei* yielded a serum which was not more active than the first, and the serum of this rabbit failed to protect when it was mixed with the virus at the time of the inoculation of the experimental mice.

The authors find that the method is of no value, and they point out that for their experiments on eight mice and four rabbits more than a hundred large rats had to be killed for the provision of the trypanosomes.

According to BRAUN and TEICHMANN *T. brucei* does not contain any toxin and here again the authors' observations are at

variance with those of Braun and Teichmann. Brief details are given of two experiments in which the intraperitoneal inoculation of two mice with 6 cg. of dried *T. brucei* caused marked symptoms of ill health with hypothermia in one and death with a lowering of the temperature of 5 degrees in the other. There was no peritoneal haemorrhage to account for the animal's death.

(410) von Schuckmann (W.) & Wernicke (K.). **Einiges über Methoden und Ergebnisse der Trypanosomenzüchtung.** [Methods and Results of Trypanosome Cultivation.]—*Centralbl. f. Bakt.* 1. Abt., Orig. 1913. Mar. 1. Vol. 68. No. 2. pp. 241-255. With 1 text-figure.

In this paper are described various modifications of the Novy-McNeal method of cultivating trypanosomes. Attempts were made to replace the rabbit blood by blood from other animals and also by using mixtures of serum from one species with corpuscles from another. The method was further modified in that the tubes of media were sterilised by heat after the processes of preparation were complete, in order to destroy any contamination that might have gained access during the defibrination of the blood.

A table is given in which the results of sixty experiments are set out. This table shows the nature of the medium, its age, the number of tubes inoculated, the results, the period for which the cultures remained alive, and the number of generations through which each was carried.

For the majority of the experiments bird trypanosomes were used.

It was found that no particular blood or serum was especially favourable for the cultivation of the parasites.

Details are also given in tabular form of a large number of culture experiments made to control the correctness of Schaudinn's view regarding *Haemoproteus* and *T. avium*. The authors were unable to obtain any evidence in support of his conclusions and they are unable to agree with the view expressed by Mayer who claimed to have proved the accuracy of Schaudinn's observations.

(411) Ponselle (A.). **Recherches sur la Culture *in vitro* du Trypanosome de l'Anguille (*Trypanosoma granulosum* Laveran et Mesnil 1902). Une Nouvelle Modification au Milieu de Novy et MacNeal.**—[The Cultivation *in vitro* of the Trypanosome of the Eel.]—*Compt. Rend. Soc. Biol.* 1913. Feb. 21. Vol. 74. No. 7. pp. 339-341.

Referring briefly to the various publications regarding the cultivation of this trypanosome the author states that he has tried the NNN medium and the heated blood medium of Mathis, but has never obtained more than a prolonged preservation of the parasite as it occurs in the blood, without any indication of multiplication. In one instance the parasite was maintained for

more than a month. The nutritive value of the media tried has been determined in each case by making cultures of *T. lewisi*.

A modification of the NNN medium has however been found in which *T. granulosum* grows rapidly, and in which the forms observed in the intestine of *Hemiclepsis marginata*, the intermediate host, have been developed.

The composition of the medium is as follows:—

Unwashed agar ...	...	...	20 grammes.
Ordinary water ...	...	...	1,000 cc.

The agar is melted and filtered and distributed to tubes in quantities of 2 to 3 cc. and then sterilised. After cooling to 50° C. an equal volume of defibrinated rabbit blood is added and the medium allowed to set in the slanting position. The culture medium is the water of condensation.

The feature of the medium is the omission of sodium chloride, rendering it very hypotonic as compared with the blood of vertebrates. The presence of 4 per 1,000 sodium chloride does not absolutely inhibit growth, but very few of the trypanosomes divide.

(412) Nattan-Larrier (L.). **Contribution a l'Étude de l'Action de la Bile sur les Trypanosomes.** [The Action of Bile upon Trypanosomes.]—*Bull. Soc. Path. Exot.* 1913. Jan. Vol. 6. No. 1. pp. 24-28.

The experiments were carried out with the acentrosomic variety of *T. brucei* (Werbitzki), and mice were used in the majority of instances. The trypanosomes were obtained by centrifuging citrated blood, removing the trypanosome layer, and washing with salt solution. The bile was, in most cases, fresh ox bile which had been passed through a Chamberland filter. In the immunisation experiments, however, desiccated bile was used, the bile being dissolved in salt solution. The "virus" used was the sediment of washed trypanosomes mixed with an equal volume of salt solution.

In the first series of experiments the action of bile upon trypanosomes was tested.

A series of tubes was prepared by mixing two drops of "virus" with ten of salt solution and then adding bile to each tube, the first receiving two drops, the second three, and so on. A control tube was kept (the first tube in the author's series).

Preparations were made from each of the tubes after an interval of 15 minutes.

No motile trypanosomes could be seen by direct examination of a preparation from the first tube. In the other tubes, up to the seventh, the results were found to be "doubtful," while in the seventh, eighth and ninth no trypanosomes could be seen at all.

The contents of tube 1 proved fatal by intraperitoneal inoculation into a mouse. The contents of the ninth tube of the series failed to infect. The results of inoculation with material from the other tubes of the series are not given.

In the second experiment, blood containing a large number of trypanosomes was placed between a slide and cover-glass, a 60 per cent. solution of bile placed at one edge of the cover-glass, and the slide set aside at room temperature for 15 minutes. At this interval the trypanosomes were found to be still motile and apparently normal. After 20 minutes the movements were slowing down and after a further five minutes all movement had ceased, and the trypanosomes had a large posterior vacuole. After half an hour's exposure to the bile the outlines of the trypanosomes were ill-defined, and shortly after the parasites appeared granular and shrunken.

The third experiment reproduced the second save that the preparations were fixed and stained with Giemsa.

In the first preparation the majority of the trypanosomes were well stained, but some were swollen and stained of a faint pink colour. In the subsequent preparations various stages of degeneration were observed, until in the sixth the flagellum was practically all that remained, there being a few chromatin-like granules adhering to it, which no doubt represented the last remains of the cytoplasm and nucleus.

The second series of experiments was directed towards the treatment of trypanosomiasis by means of bile.

By experiment it was found that the toxic dose of bile for a mouse weighing 20 grammes was about 0·5 cc.

In this series of experiments the trypanosome of surra was used. A control mouse died on the eighth day. A second mouse was inoculated on the seventh day after infection, when trypanosomes were "not scanty" in its blood, with 0·5 cc. of bile. The mouse died two hours later, trypanosomes being present up to the time of death.

The third mouse received twice the dose of bile, and died in three hours, the trypanosomes again persisting up to the time of death.

In a second experiment a mouse in whose blood trypanosomes were scanty was given 0·5 cc. of bile. Trypanosomes were not again observed in its blood during the day, but the animal died on the following day.

In the third series of experiments an attempt was made to immunise mice by administering successive doses of a mixture of "virus" and bile, which had been incubated at 38° C. for 15 minutes, the quantity of bile in the successive doses decreasing. In no case was it possible to show that the mice acquired any immunity.

(413) Holmes (J. D. E.). **Salvarsan in the Treatment of Surra in Horses.**—*Memoirs of the Dept. of Agric. in India, Veterinary Series.* 1913. Vol. 1. No. 2. pp. 89-107.

In preliminary experiments the toxic dose by intravenous injection was ascertained approximately, and the effects of both small and full sub-toxic doses were gauged by the period for which the peripheral circulation remained free from trypanosomes after treatment.

In the first series of experiments 17 animals were given a single dose of the drug. Of these three recovered, 11 had relapses, and three died. The dose administered varied from ·0014 to ·0094 grammes per pound body-weight.

In the second series of experiments three injections were given at intervals of one day. Only two animals were treated in this manner; in one there was a relapse and the other remained under observation for ten months without a reappearance of the trypanosomes being recorded.

Two animals received three injections at intervals of one week. In both cases there was a relapse. Four animals were given, in addition to the three injections of salvarsan, one dose of arsenic *in bolus*, the dose being 1 gramme. In none of these was a cure effected.

The author's conclusions may be summarised as follows:—

1. The toxic dose of salvarsan for the horse administered intravenously is approximately ·01 gramme per pound body-weight, but individual susceptibilty exists.

2. An intravenous injection of salvarsan is followed by a disappearance of trypanosomes from the peripheral circulation for periods varying from 6 to 36 days. The relationship between the dose of the drug and the period of absence of the trypanosomes is neither regular nor definite.

3. In three cases a single dose effected a cure.

4. By repeated administration a tolerance to the drug is established.

5. Three intravenous injections did not give results superior to those obtained by a single injection.

6. The administration of a large dose of arsenious oxide followed 24 hours later by an intravenous injection of salvarsan produced no better results than a single dose of either.

(414) Holmes (J. D. E.). **Salvarsan in the Treatment of Surra in Dogs.**—*Memoirs of the Dept. of Agric. in India, Veterinary Series.* 1913. Vol. 1. No. 2. pp. 109-146.

The difficulties in the way of treating dogs for surra lie in the facts that the dog is particularly susceptible to the disease and also very intolerant of the drugs which have given the most encouraging results in other species.

Twenty-nine dogs were given a single injection of salvarsan in doses varying from ·006 to ·063 grammes per pound body-weight.

Of these two remained under observation for 370 days without relapse. Eleven died in periods varying from a day to several weeks after treatment, but for the most part trypanosomes were absent from the circulation during that time. In four cases there were relapses, and the remainder of the dogs had to be destroyed before the experiments were complete owing to their having come into contact with a dog suspected of rabies.

In all these cases the drug was administered in a 0·5 per cent. solution.

In six instances the drug was used in a 1 in 250 solution, but all the dogs died within 48 hours after the injections were given.

Six dogs received three injections at intervals of a day, but either death or relapse occurred in each case.

Of two dogs which received three injections at intervals of a week one recovered and one died. Similar results attended the experiments when only two injections were given at a similar interval.

Three dogs were given a single subcutaneous injection but none recovered. It was found that subcutaneous injection produced painful swellings which either burst or were absorbed.

Intramuscular injection proved successful in one case and failed in another.

The following conclusions are drawn:—

1. The toxic dose of salvarsan for the dog is about ·025 gramme per pound body-weight, and ·02 gramme is fairly well tolerated.

More dilute solutions produce more markedly toxic symptoms than more concentrated ones.

2. An intravenous injection may cause the disappearance of the trypanosomes from the circulation for periods varying from 20 to 97 days.

3. Repeated injections do not give better results than a single administration of the drug.

4. Subcutaneous and intramuscular injections are not well borne. Three rabbits which were given doses of ·000042 to ·000064 gramme intravenously all recovered.

(415) Salmon (P.). **L'Acridine dans le Traitement de la Maladie du Sommeil expérimentale.** [Acridine in the Treatment of experimental Sleeping Sickness.]—*Compt. Rend. Soc. Biol.* 1913. Jan. 24. Vol. 74. No. 3. pp. 134-136.

A number of observers have recorded the effects produced by certain dyes of the acridine or oxazine series upon trypanosomes. There is vital staining of the blepharoplast, followed by disappearance of this structure, and destruction of the parasite.

During the course of a number of experiments regarding the process by which these changes are produced the author has obtained some favourable results in the treatment of trypanosomiasis, and especially with *T. gambiense*.

The strain of *T. gambiense* used was one which constantly proved fatal to mice in six days. The dye which gave the best results was Trypoflavin A (acridine) administered subcutaneously.

A dose of 0·06 mg. was found to protect a mouse when injected simultaneously with infective blood. In one experiment, in which the curative action of the dye was tested, four mice showing numbers of trypanosomes in their blood were injected with doses varying from 0·25 to 0·04 mg. and all save the one which received the smallest dose recovered. The latter still showed trypanosomes in its blood on the seventh day of the disease. The control mouse died on the sixth day.

Experiments showed that there is a large margin between the toxic and the therapeutic dose, the proportion being about 5 to 1.

If, however, the toxicity of this dye be compared with that of the various anilin dyes, trypoflavin falls into the class comprising the dyes of fairly high toxicity; care should therefore be taken in its application.

The destruction of the parasites occurs slowly. Trypanosomes may be found in the blood several hours after the injection. This is somewhat different to what happens in the case of arsenical compounds, which destroy trypanosomes within an hour.

A point in favour of acridine as compared with such substances as trypanblue is that it does not cause general colouration of the tissues; it produces, however, a yellow fluorescence in the urine.

(416) Coles (A. C.). **Trypanosomes found in a Cow in England.**—*Parasitology.* 1913. Jan. Vol. 5. No. 4. pp. 247-252. With 1 plate.

After summarising the literature that has appeared on the occurrence of trypanosomes of the *theileri* type in the blood of cattle, the author describes two specimens found by him in the blood of a cow in Dorsetshire.

At the time when the trypanosomes were found the animal was the subject of redwater, *Babesia bovis* being present in the blood and about 2 per cent. of the blood corpuscles invaded. Two parasites were found in one blood film out of seven examined, and none were found in 20 films made two days later.

The trypanosome measured 98 microns in length including the flagellum. The body measured 88; the flagellum 10; from the posterior extremity to the kinetonucleus 37, and to the centre of the trophonucleus 44; the diameter of the body 6; and the width of the undulating membrane 3·5 microns.

(417) Cazalbou (M. L.). **Observation d'un nouveau Trypanosome chez le Lapin.** [A new Trypanosome of the Rabbit.]—*Recueil de Méd. Vét.* 1913. Mar. 15. Vol. 90. No. 5. pp. 155-158.

The following details are given of an outbreak of disease occurring among some young rabbits at Rennes.

The animals when about three weeks old suddenly ceased to grow, rapidly lost condition, became affected with paralysis of the hind limbs, and died after a few weeks. The appetite remained good throughout.

Examination of the blood revealed the presence of a trypanosome, a single specimen being found in 15 blood smears.

The parasite was a very large one measuring about 80 microns in length, and having a free flagellum measuring 10 to 12 microns. The body at its widest part measured 8 microns, and an undulating membrane was distinctly visible opposite this wide portion of the body only. The nucleus was central and oval in shape. It appeared to be composed of homogeneous protoplasm.

The author failed to infect guinea-pigs by inoculation, and circumstances prevented him from inoculating rabbits, so that he was unable to establish the pathogenicity of the trypanosome. A number of references are given to records of the occurrence of trypanosomes in the rabbit.

SPIROCHAETOSIS.

(418) Danulesco (V.). **Essais de Culture du Spirille de la Poule.** [The Cultivation of the Spirochaete of the Fowl.]—*Compt. Rend. Soc. Biol.* 1913. Feb. 28. Vol. 74. No. 8. pp. 369-371.

The author has successfully applied the medium described by Noguchi to the cultivation of the Spirochaete of the fowl.

The infective blood is mixed with an equal volume of 1·5 per cent. citrate solution and distributed in quantities of about 10 drops to tubes in which have been placed fragments of fresh rabbit kidney. Ten or fifteen cubic centimetres of unfiltered sterile ascitic fluid are then added and finally a layer of sterile vaseline. The tubes are incubated at 37° C. Absolute asepsis is essential.

Four samples of ascitic fluid were used in the experiments and one was found to be far superior to the other three.

The author has found that in citrated blood maintained at 37° C. the spirochaetes will remain alive for 24 to 36 hours, for 48 hours in broth containing a fragment of fresh tissue, slightly longer in simple ascitic fluid or ascitic agar, and for fifteen to twenty days in the medium described.

In the latter medium the multiplication of the parasites is rapid. Within twenty-four hours there may be 15-20 spirochaetes in each field of the microscope where immediately after inoculation only four or five could be seen. During the following three days the number may be more than doubled. The spirochaetes preserve their motility and their general appearance save that they are as a rule a little shorter than those occurring in the blood of an infected bird. During the first day the parasites are present in all parts of the liquid and especially around the fragment of kidney. Gradually they begin to collect into little masses, and as the cultures get older show a tendency to pass into the upper layers of the liquid.

From the fifth day, and sometimes even earlier, some of the spirochaetes present a deformed and irregular appearance, and they may also appear to be granular. The granular parasites have lost their motility. In the majority of the tubes the parasites are all dead by the twelfth day, but sometimes they survive until the 15th day and in one case a culture was still alive on the 20th day.

Subcultures have been made using as seed material cultures from four to seven days old. The author has succeeded in obtaining five generations of subcultures. In the later generations an increasing number of the tubes proved sterile and there was a steady decrease in the abundance of the growth.

(419) Launoy (L.) & Levaditi (C.). **Nouvelles Recherches sur la Thérapeutique Mercurielle des Spirilloses (Sp. des Poules et Syphilis du Lapin).** [New Investigations regarding the Mercurial Treatment of the Spirochaetoses.]—*Compt. Rend. Soc. Biol.* 1913. Jan. 10. Vol. 74. No. 1. pp. 18-21.

The group of compounds investigated by the authors in these experiments were the dinitro and diamino compounds of the mercury salt of dioxydiphenyl.

The toxicity and the instability of the mercury salt of dioxydinitrodiphenyl and of dioxydiaminodiphenyl rendered them unsuitable, and the diacetyl derivative of the second of these substances was the only one found to be of any value.

For the sake of convenience this substance is called No. 114. It contains 42·8 per cent. of mercury and the solution used was prepared by mixing 0·2 g. of substance 114 with 2 cc. of normal sodium hydrate and 18 cc. of normal salt solution.

For the rabbit the toxic dose by intravenous injection was found to be 0·04 to 0·05 per 1,000. The toxic dose was that which produced a fatal result in 15 to 20 days. The toxic dose for the fowl was practically the same.

It was found that the drug exercised a protective effect when administered intravenously in doses of 0·04 per 1,000 within 24 hours after infection. The curative effect is less marked, and the drug appeared to be more toxic for birds treated when parasites were numerous in the blood, owing to decreased resistance caused by the infection.

(420) Gleitsmann. **Beitrag zur Entwicklungsgeschichte der Spirochäten (Borrelien).** [The Life-history of Spirochaetes (Borrelia).]—*Centralbl. f. Bakt.* 1. Abt., Orig. 1913. Feb. 12. Vol. 68. No. 1. pp. 31-49. With 1 plate and 2 text-figures.

After giving a somewhat lengthy review of the literature, the author describes his own investigations regarding the life history of the organisms, special attention being paid to the so-called "intra-corpuscular bodies" (Balfour).

Two strains were used, viz., Marchoux's Brazilian strain and the Sudanese spirochaete of Balfour.

The first series of experiments was carried out *in vitro* and the immunity reactions of the two spirochaetes with the homologous and the heterologous immune sera investigated.

The sera were added to the spirochaetes in dilutions from ½ to 1/200, and it was found in each case that the homologous serum exerted a far more powerful action on the parasites than the heterologous. A polyvalent serum tested with each of the spirochaetes exerted the same effect upon both.

Experiments carried out upon fowls indicated that in spite of the results obtained *in vitro* the parasites must be closely related since a fowl immune to one strain was also immune to the other. In these experiments fowls were inoculated with mixtures composed of blood containing the parasites and the homologous and heterologous immune serum. The birds remained healthy.

In a further series of experiments fowls were infected by means of ticks and the survivors reinfected with the other strain. In each case immunity had been established by the first infection.

The author then passes on to a consideration of Balfour's "intra-corpuscular bodies," specimens being examined with dark ground illumination and also in films stained in the manner recommended by Balfour. He rather favours the view that these bodies do not in reality represent a stage in the life cycle of the

spirochaete, but that they are degeneration products of some kind; although the possibility is admitted that they may represent some separate infection.

Tabulated details are given of the course of infection in fourteen birds. Three birds infected with the Sudan strain and one with the Brazilian strain showed chronic symptoms, but in no instance was the relapse associated with the presence of spirochaetes or intra-corpuscular bodies.

When the parasites were numerously present in the circulation the nuclei of the red corpuscles underwent a process of karyolysis and fragments which stained deeply were separated off. Spirochaetes were also observed to penetrate into red corpuscles without becoming altered in appearance.

LEISHMANIASIS.

(421) Row (R.). **Some Experimental Facts *re* Kala-azar (Indian). Second Memoir.**—*Jl. Trop. Med. & Hyg.* 1913. Jan. 1. Vol. 16. No. 1. pp. 1-2.

In a previous communication the author has published the results obtained by inoculating *Macacus sinicus* (see this *Bulletin*, 1913, Vol. 1, No. 3, Abst. No. 309, p. 173), and in the present paper records some further observations on this and other species of animals.

The monkey originally infected developed two lesions about the size of beans at the seats of inoculation after 5½ months. One of these was excised a fortnight after its appearance, and an emulsion made from a portion of it was used for the inoculation of fresh animals. Microscopic examination of the emulsion showed that it was not rich and that therefore the doses administered were very small. Two mice and a *Macacus sinicus* were inoculated intraperitonally with the emulsion, and one mouse with a mere trace of juice obtained by puncture of one of the lesions of the first infected monkey. Two of the mice were killed six weeks and two months after inoculation and the third died in six weeks. In each case microscopic examination showed that there had been a generalised infection. The liver of the monkey was aspirated four and a half months after infection and the parasite was found in the blood in small numbers, both free and in macrophages.

The liver and spleen of the mouse that died were removed 8 to 10 hours after death and an emulsion made from them was injected intraperitoneally into a fresh mouse. When this mouse was killed six weeks later no evidence of infection could be found.

The livers and spleens of the other two mice were treated in the same way and in each case two fresh mice were inoculated. In none of these animals was any definite evidence of infection found, and the author thinks that these negative results may possibly be attributed to one of the following causes:—

1. Possibly the animals were examined too soon, although fully seven to nine weeks were allowed in each case.

2. The possibility of the attenuation of the virus by passage through mice.

3. The possible curative effect of the extract of the liver and spleen introduced simultaneously with the parasites.

In addition to the above results the author records the production of a local pinhead nodule in a *Macacus sinicus* inoculated cutaneously from the monkey mentioned in his first paper already referred to. The incubation period was five months. The nodule contained typical parasites which yielded flagellates in cultures.

Dogs, rabbits, guinea-pigs, and wild mice have been found refractory to small doses of the virus given intraperitoneally and to large doses of culture given intraperitoneally or subcutaneously.

(422) Gray (A. C. H.). **Leishmaniose naturelle du Chien à Tunis.** [Natural Leishmaniasis of the Dog in Tunis.]—*Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 165-166.

Examinations made by other authors have shown that the proportion of infected dogs in Tunis is 1·6 per cent. The present author has examined 127 dogs and has found two infected (1·6 per cent.).

In one animal the parasites were numerously present in the bone marrow but scanty in the spleen and liver. Cultures were obtained on NNN medium, but inoculation into two dogs failed.

In the second dog parasites were very numerous in the bone marrow, spleen and liver. They were also found in the portal blood. Cultivation failed owing to the presence of impurities.

(423) Lignos (A.). **L'Infection par Leishmania des Chiens de l'Ile d'Hydra.** [Canine Leishmaniasis in the Island of Hydra.] —*Bull. Soc. Path. Exot.* 1913. Feb. Vol. 6. No. 2. p. 117.

It would appear from the statistics that the Island of Hydra is more extensively infected with kala azar than any other country in which the disease occurs. The total population of the Island is about 6,000 and the annual number of births about 120. It is found that one child in ten becomes affected.

In view of the facts, established by Nicolle, that the disease occurs in the dog and that children can contract infection from that animal, an examination of the dogs in the Island was undertaken to ascertain in what proportion these were affected.

During the summer of 1912, 48 dogs were examined, this accounting for half the dogs on the Island. As the inhabitants objected to the destruction of their animals the examinations had then to be brought to a close.

Eight (16·66 per cent.) of the dogs were found to be infected. The distribution of the cases during the months May to October was as follows:—

	Dogs examined.	Infected.
May	5	1
June	16	2
July	8	1
August	10	1
September	7	2
October	2	1

(424) Laveran (A.). **Infections du Cobaye, du Lapin et du Chat par la *Leishmania infantum*.** [The Transmission of *Leishmania infantum* to the Guinea-pig, Rabbit, and Cat.] — *Bull. Soc. Path. Exot.* 1913. Feb. Vol. 6. No. 2. pp. 110-114.

This article is a review of the various attempts that have been made to infect guinea-pigs, rabbits, and cats experimentally with *L. infantum*.

Guinea-pigs.—In 1909 the author in conjunction with Pettit shewed that the presence of the parasite in the peritoneum of guinea-pigs 59 days after intraperitoneal inoculation could be proved by cultural methods, flagellates being developed on NNN medium.

In 1911 Franchini succeeded in producing a general infection in a young guinea-pig by intraperitoneal inoculation with 1 cc. of a fifteen-days-old culture of the eighth generation. The guinea-pig was killed on the 26th day. Parasites were found in smears from the spleen, liver, bone marrow, and blood, but more numerously in the spleen than elsewhere.

Nicolle and Blaizot have failed to produce infection in spite of repeated attempts.

The author failed in the case of two guinea-pigs inoculated in the liver with material obtained from an infected dog, in a guinea-pig inoculated intraperitoneally with peritoneal serous fluid from an infected guinea-pig, and in four guinea-pigs inoculated in various ways with large doses of culture. In a single instance there was a slight localised infection of the peritoneum in a guinea-pig inoculated intraperitoneally with material from an infected dog.

Rabbitt.—In 1911 Volpino published an account of a successful infection of a rabbit. The infection was achieved by placing some infective bone marrow from a dog upon the scarified cornea. Three months later there was opacity of the cornea and typical parasites could be found in large mononuclear leucocytes.

Mantovini produced a general infection by intravenous inoculation with a culture. The animal was killed 20 days after inoculation, and cultures were obtained. The progress of the infection in this case was very rapid.

Nicolle, Blaizot and Laveran have failed to set up injection on a number of occasions.

Cat.—Apparently the only recorded case of natural infection in a cat is that described by Ed. and Et. Sergent, Lombard, and Quilichini. In this instance a child, a dog, and a kitten aged four months in the same house were found to be infected.

Nicolle examined 51 cats from Tunis and Gafsa without finding a single one infected. The same author inoculated four cats unsuccessfully. Laveran has made several attempts to infect cats but without success. Nicolle and Blaizot failed to infect a cat by intravenous inoculation with growth from six tubes of culture.

While examining preparations made from fleas caught on cats inoculated but not infected with Leishmania, Laveran found large numbers of parasites bearing a close resemblance to Leishmania.

The organisms were round or oval in shape and measured from 2 to 6 microns in length, by 2 to 4 in width. In many cases they were grouped into clusters. The cytoplasm, which stained a faint blue with Giemsa, contained a round or oval principal karyosome and an accessory rod-like karysome. Flagellated forms were not seen, but the author thinks that there can be no doubt that the parasite is related to the trypanosomides described by a number of authors as occurring in other species of flea.

(425) Bandi (I.). **Preliminary Note on the Identity of certain Leishmaniases based upon Biological Reactions.**—*Jl. Trop. Med. & Hyg.* 1913. Feb. 15. Vol. 16. No. 4. p. 50.

The primary object of the author's investigations was to ascertain whether a method of diagnosis based upon biological reactions could be devised.

In the first series of experiments an attempt was made to ascertain whether and in what measure specific substances are elaborated in the blood of animals inoculated with cultures of Leishmania. Two strains were used, one of *L. infantum*, and one of canine origin, both of which were obtained from Nicolle.

Rabbits were used for the inoculation experiments.

The author has observed that repeated intravenous inoculations with cultures, while not giving rise to any pathological condition, induce the formation in the blood of specific substances which agglomerate the parasites, and which can be compared to bacterial agglutinins. The serum of inoculated rabbits shewed this property in dilutions up to 1 in 200. The serum of normal rabbits had no action.

The serum of rabbits inoculated with canine leishmania agglutinated both this parasite and *L. infantum* in dilutions up to 1 in 160. Sera of rabbits inoculated either with canine Leishmania or *L. infantum* agglutinated *L. tropica* in dilutions up to 1 in 70 only.

The fact that the serum of rabbits inoculated with *L. infantum* and with the canine strain agglutinate both the homologous and the heterologous parasite to the same extent while they agglutinate *L. tropica* to a much lower degree shews that there is a general group reaction, and also tends to support the view that the parasites of the disease in the dog and child are identical.

PROTOZOA.

(426) Nicolle (C.) & Conor (M.). **La Toxoplasmose du Gondi. Maladie Naturelle.—Maladie Expérimentale.** [Toxoplasmosis of the Gondi—Natural and Experimental.]—*Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 160-165.

In May 1912 the authors received a batch of Gondi from Matmata in Southern Tunis where the parasite was originally found in 1907 and again in 1908.

At the time of their arrival the animals had on them two species of ectoparasites,—an undetermined Ixode and a larval Trombidium of undetermined species. They were immediately freed

from these parasites. During the two months following their arrival half the animals died, and although a post-mortem was made in every case, Toxoplasma was not found. The mortality ceased in July, but the animals commenced to die again at the end of September, and by the beginning of January nine more were dead. Eight of these were found to be infected with Toxoplasma. Subsequently two more died but these were not affected.

On post-mortem the animals were generally found to be in fat condition, but in the majority of cases there were obvious lesions: a clear bilateral pleural exudate associated with congestion or hepatisation of the lungs, enlargement of the spleen up to three or four times, and occasionally slight enlargement of the liver.

The parasite was present in enormous numbers in smears from the spleen, less abundant in the liver and lungs, rare in the kidneys, and exceptional in the blood and bone marrow.

The parasites varied in shape and measured on an average 5 by 2·5 microns.

The nucleus was single and generally centrally placed. In those parasites in which it was placed towards one end that end appeared swollen, the other end being in many instances drawn out.

The parasites occurred either free or enclosed in cells. In the latter case mononuclear leucocytes were more frequently invaded than polynuclear leucocytes. Multiplication forms were very numerous, and the only form of multiplication observed was longitudinal division, the process starting at the nucleus. Whether intracellular or free, groups of parasites numbering as many as 20 may be seen and such groups generally have a rounded outline suggesting encystment. The authors do not agree with Splendore that there is actual encystment for the following reasons:—They have never observed the stage preceding that in which every toxoplasma is individually recognisable, they have been unable to make out any envelope, and, save for differences in size, masses of parasites resemble each other exactly.

The authors have succeeded in infecting two gondis experimentally by intraperitoneal inoculation with bone marrow from an infected animal, the material used being very poor in parasites. The periods of incubation were eight and nine days.

The mouse is said by the authors to be more susceptible than the gondi, death taking place in five or six days after intraperitoneal inoculation. In the mouse, however, the infection is not a general one, the parasites being practically limited to the peritoneal exudate, of which there is a large amount, and to the endothelium of the peritoneal membrane.

In two cases the infection was successfully transmitted by intramuscular inoculation, the period of incubation being about a week.

Nicolle and Manceaux have already recorded one instance in which a guinea-pig was successfully infected experimentally. The present authors have had positive results in four out of seventeen animals.

Infection has only followed intraperitoneal inoculation and this fails unless young guinea-pigs are used. The period of incubation was four to six days. At the post-mortem there was found peritonitis with abundant exudate serous and fibrinous containing numerous parasites. There was an occasional organism in the liver and spleen, but none were found in the bone marrow and the blood.

The pigeon is susceptible to infection, but less so than the mouse, and the course of the disease is different.

Infection in the pigeon is slower and has a greater tendency to generalisation. Intraperitoneal inoculation is the only method of setting up infection. Of eighteen birds inoculated eleven died in the nine to thirty-one days, parasites being demonstrated in each case.

Parasites are present in the liver, in which they may be found in enormous numbers, spleen, lungs, bone marrow, kidneys and blood.

Toxoplasma was not present in the peritoneal false membranes save in those birds which died within a short period.

The authors have failed to infect rabbits, rats, a dog, monkeys, toads and frogs, and they have been unable to cultivate the parasite outside the body.

(427) Laveran (A.) & Nattan-Larrier. **Au Sujet des Altérations Anatomiques produites par le *Toxoplasma cuniculi*.** [The Lesions produced by *Toxoplasma cuniculi*.]—*Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 158-160.

The authors have studied the macroscopic and microscopic appearances of the lesions in the liver, spleen, and lungs, in pieces of tissue received from Splendore.

The spleen is markedly enlarged and the liver presents an appearance suggestive of acute miliary tuberculosis. The lungs have a number of greyish nodules scattered through them.

The lesions in the liver vary in size up to one millimetre in diameter, and are scattered throughout the organ. In the early stages the lesions are rounded, but later have rather ill-defined margins. Under the microscope it is seen that in the earliest stages there is a loss of detail and staining affinities on the part of the affected liver cells, the cells becoming necrotic. There is no influx of leucocytes into the lesion. In the neighbouring capillaries there is thrombosis and proliferation of the endothelial cells. The centre of the lesion becomes converted into a kind of fibrinous network in the meshes of which are to be seen nuclear fragments and large numbers of red corpuscles. Around this central portion there is an irregular zone in which the outlines of the cells are still distinct, but in which the endothelial cells of the capillaries are profoundly altered.

By means of ordinary staining methods the distribution of the parasite can be easily followed, the liver cells being to a large extent invaded. Eight to ten parasites may be frequently found in cells that appear to be quite normal, but others containing 20 to 30 parasites are in the early stages of necrosis. The neigh-

bouring capillaries may be normal or impermeable. In the latter case they contain immense numbers of parasites, but the endothelial cells are not invaded. The organisms vary from 20 to 25 microns in diameter, and are rounded or oval in shape. They contain a variable number of karyosomes. Toxoplasma is not to be found in the central portions of the lesions.

The lesions in the spleen and lungs have the same essential features as those in the liver.

(428) Rodhain (J.), Pons (C.), Vandenbranden (F.), & Bequaert (J.). **Note sur des Trypanosomides intestinaux d'Haematopota au Congo belge.** [Intestinal Trypanosomides of Haematopota in the Belgian Congo.]—*Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 182-184. With 1 text-figure.

The authors have encountered trypanosomides in the intestinal canal of two species of Haematopota.

Two flies out of a batch of 11 *Haematopota duttoni* (Newstead) were found to have flagellates and cysts in their rectum. The motile organisms resembled those described by Bruce as parasites of *Tabanus secedens*. The cysts which were oval or pyriform presented an appearance similar to the spermoides described by Chatton.

A quite different flagellate was found in *Haematopota vandenbrandeni* at Sankisia. In this instance only one fly out of 39 was found to harbour the parasite. Cysts were also found in this case.

The flagellates took the form of slender elongated crithidiae, the posterior extremity of which was drawn out into a point, the anterior portion carrying an undulating membrane closely applied to the body.

The largest parasites measured from 45 to 50 microns in length, a free flagellum of 16 to 18 microns being included in this measurement. The body was between 1·5 and 2 microns in thickness at the level of the nucleus, which was situated at a point about 10 to 14 microns from the anterior end of the body. There was no distinct karyosome, but the nucleus contained a number of irregular granules of chromatin.

The blepharoplast was punctiform and was placed about four microns in front of the nucleus, and the flagellum was relatively thick.

Posterior to the nucleus there were often observed in the cytoplasm a number of metachromatic granules.

In addition to the large flagellates there were smaller forms measuring only 31 to 39 microns in length.

Between the crithidia provided with undulating membrane and flagellum and the cysts, there was a series of gregarine-like bodies with short flagella.

The cyst forms were about six microns by two to four microns and they were surrounded by a delicate eosinophile layer. One extremity was slightly larger than the other and contained the nucleus, just posterior to which was the blepharoplast.

Non-flagellated forms were also observed which appeared to represent the stage preceding cyst formation.

The name *Crithidia tenuis* is suggested for the flagellate found in *H. vandenbrandeni* of which it is probably a parasite proper.

(429) Chatton (E.) & Roubaud (E.). **Sporogonie d'une Hémogrégarine chez une Tsétsé (*Glossina palpalis* R. Resv.).** [Sporogony in a Haemogregarine in *G. palpalis*.]—*Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 226-233. With 2 plates.

At Kolda on the Casamance the authors have encountered in the body cavity of *G. palpalis* the cysts of an unidentified haemogregarine. These forms have not been observed elsewhere nor in any other fly. Out of a total of 465 flies only four were found to be infected, and it should be noted that the intestinal tract of these four flies also contained *T. grayi*. The flies when fasting were distinguishable from others by the fact that the abdomen was slightly swollen, and on making an incision there was found a mass of whitish powdery material composed of rounded grains of different sizes. The largest had a mulberry-like appearance and contained a number of secondary cysts which in turn contained numerous sporozoites. Examined in salt solution and after slight pressure had been exerted vermicular bodies capable of exercising slow movements were found to escape. These bodies exactly resembled haemogregarines as they occur in the blood. The larger cysts were situated immediately under the chitinous covering of the body, and in the deeper parts among the loops of the digestive canal mature sporozoites liberated from the large cysts were to be found. Parasites were not found in the thorax, but both cysts and sporozoites were found in the head. The wall of the intestine and the salivary glands were free from parasites.

The authors have been able to follow the development of the sporozoites from the sporoblasts, but as yet they have not traced any copulation.

The youngest stage that they have observed is a rounded body measuring from 50 to 70 microns. This is surrounded by a thin cyst membrane and contains a large nucleus provided with a karyosome. They have observed only one stage of the division of the nucleus, namely the formation of about a dozen plumous chromosomes from the karyosome, but they believe that it is a case of multiple nuclear division such as is known to occur in some of the gregarines and coccidia. After nuclear division the parts of the nuclear substance become scattered throughout the cytoplasm, but soon commence to arrange themselves side by side at the periphery. At this stage the parasite measures about 100 microns.

Up to this point in its development the parasite has one remarkable characteristic, namely, that its surface and the cyst membrane are not smooth but irregular. The cytoplasm becomes vacuolated and the nuclei project from the surface, and shortly afterwards are contained in little bud-like projections attached

to the general surface of the parasite by constricted necks. These buds become detached and rapidly increase in size until they measure 10 to 12 microns. These bodies are the sporoblasts.

The nucleus of each sporoblast divides into two and after an interval into a number of portions which arrange themselves in two rows or rather in two parallel planes, and segmentation occurs at right angles to these planes. The sporoblast is covered with a resistant membrane. It is these sporocysts which form the whitish powder in the body cavity of infected flies. Isolated sporozoites have been studied in preparations stained by Giemsa. They take the form of curved vermiform bodies. The nucleus is placed at about the posterior third of the body, which is rounded at the posterior and pointed at the anterior end. In front of and behind the nucleus there are two bodies which stain of a deep violet tint with Giemsa. The anterior one is by far the larger of the two.

The view that the parasite is a haemogregarine which passes through the stage of sporogony is justified by recent investigations into the life history of a number of haemogregarines. The parasite would appear to approach most nearly to *Hepatozoon perniciosum*.

The authors are inclined to think that the vertebrate host of the parasite is a crocodile or a lizard.

(430) Joukoff (N. M.). **Culture du Parasite de la Malaria.**— *Compt. Rend. Soc. Biol.* 1913. Jan. 24. Vol. 74. No. 3. pp. 136-138. With 1 text-figure.

The authors have succeeded in cultivating *Plasmodium praecox* and *Plasmodium malariae*. Their technique is as follows:—

Ten cubic centimetres of blood containing *Plasmodium praecox* were placed in each of a number of tubes and ½ cc. of 10 per cent. sodium citrate solution was added. Some of the tubes were incubated at 41° and some at 44° C., the period of incubation in each case being half an hour.

As it was desired to produce the sporogonous forms the tubes so incubated were then incubated at 25° to 26°, since sporogony normally occurs in the mosquito at about this temperature.

Examinations were made every three hours.

No parasites could be found in the tubes which had been first incubated at 44° C. In the tubes incubated at 41° C. the first stages of the sexual cycle of the parasite were obtained. This culture survived only three days.

In the second experiment *Plasmodium malariae* was used. The tubes of citrated blood had added to them 1½ cc. to 2 cc. of Lokke serum before incubation at 41° C. In these cultures the authors observed not only conjugation but also the subsequent segmentation of the fertilised elements. On the sixth day a large number of sporozoites of various shapes were seen, some in which division had not actually occurred, and some in which it was complete.

The segmentation was different from that observed in the mosquito, but this is due according to the authors to differences in the conditions compared with those prevailing in the mosquito.

(431) Rowley-Lawson (Mary). **The Extracellular Relation of the Malarial Parasite to the Red Corpuscle, and its Method of securing attachment to the External Surface of the Red Corpuscle.** —*Jl. Experiment. Med.* 1913. Mar. 1. Vol. 17. No. 3. pp. 324-343. With 6 plates.

In this paper the view is expressed that the malarial parasite is extra-corpuscular during the whole of its developmental cycle, and that it retains its hold upon the external surface of the red corpuscle by means of pseudopodia. Numerous photographs are given in the plates in support of this view.

HELMINTHS.

(432) Brau & Bruyant (L.). **Quelques Notes sur les Helminthes du Porc en Cochinchine.** [Helminths occurring in the Pig in Cochinchina.]—*Bull. Soc. Path. Exot.* 1913. Jan. Vol. 6. No. 1. pp. 41-43.

Trematodes. In 1911 Mathis and Leger recorded the occurrence of *Fasciolopsis buski* in 6 per cent. of pigs at Tonkin; commenting at the same time on the rare occurrence of this parasite in man. They also drew attention to the statement of Barrois and Noc that the parasite was of frequent occurrence in man in Cochinchina, and expressed the desirability of examining the pigs slaughtered at Saigon (Cochinchina).

The present authors made the desired examination of 100 pigs at Saigon, but were unable to find a single specimen of the parasite.

Although *F. buski* was not found, an amphistone possessing all the characters of *Gastrodiscus* Leuckart was found in 5 per cent. of the pigs examined. Save for minute differences in preserved specimens, which the authors think were due to shrinkage, the parasite was indistinguishable from *G. hominis*.

The discovery appears to confirm the idea that, although the parasite had been found only in man, it probably occurred also in an animal, and particularly in a domesticated animal.

Cestodes. *Taenia solium* would appear to be somewhat rare in Asia and particularly in Cochinchina, but the authors have received from Donnadieu a pig's heart literally crammed with cysticerci, which on examination proved to be *C. cellulosae*.

Nematodes. Four species of nematodes have been encountered in the intestines of pigs:

Oesophagostoma dentatum in 95 per cent. of pigs.

Arduenna strongylina in 2 per cent.

Arduenna dentata in 2 per cent.

Trichuris crenatus in 3 per cent.

Only 5 per cent. of the pigs examined had no worm parasites in the alimentary canal.

(433) Mitter (S. N.). **Further Note on a Gastrodisc(?) from an Indian Zebu.**—*Vet. Jl.* 1913. Mar. Vol. 69. No. 453. p. 117. With 1 text-figure.

The author has submitted specimens of this parasite, of which he has already published a description in the Journal of Comparative Pathology and Therapeutics, to Railliet for identification. The parasite is stated to be a Homolostoger [? Homalogaster], and the author believes that his specimens are a local variety of *H. paloniae*. The figure given is an illustration taken from a paper by Benham in Lankester's "Zoology."

RABIES.

(434) Acton (H. W.) & Harvey (W. F.). **The Fixation of Rabies Virus in the Monkey (*Macacus rhesus*) with a Study of the Appearance of the Negri Bodies in the different Passages.**—*Parasitology.* 1913. Jan. Vol. 5. No. 4. pp. 227-233. With 1 plate.

In the brief introductory portion of this paper reference is made to the opinion held by Magendie that the rabies virus when transmitted by bite from dog to dog lost its virulence about the fifth passage. This view was also held by Celli and Marino-Zucco who considered that they had confirmed it by experiments.

In a recent experiment the authors have found that on the passage of a fixed virus of the rabbit through a dog the virus remained fixed for the dog, but in the fifth passage the animal did not show rabies. When this dog was re-inoculated 20 days later with material from the brain of the fourth dog in the series of passages it contracted dumb rabies after the fixed incubation period, thus showing that the loss of virulence was not due to attenuation, but to technique. They therefore hold that in all experimental work on rabies the controls should be equal in every respect to the test animals.

In *Macacus rhesus* the authors have found that the virus of rabies becomes fixed just as in the rabbit.

In the first two passages in this species the rabies was of the furious type; from the third to the sixth passage the symptoms commenced with fury, but for the last 24 to 48 hours there was complete paralysis. From the seventh passage onwards the paralytic type was alone observed.

In the monkey from the fourth passage onwards the incubation period varied from 7 to 8 days. The incubation period in rabbits inoculated from the monkeys showed a fixed virus period of incubation from the seventh passage onwards.

A converse experiment was also made to show that the fixed virus of the rabbit, when passed through *Macacus rhesus*, is not altered in its virulence either for this monkey or for the rabbit.

The formation of negri bodies.—

The results of the authors' study of Negri bodies in the ganglion cells of the hippocampus of *Macacus rhesus* have confirmed them in their view that they are nothing more or less than developments round nuclear fragments.

In each monkey a hundred ganglion cells were examined and the size, number, and colouration of the cell inclusions noted. The sections were stained with Mallory's iron haematoxylin and Bordeaux red.

The results of these examinations are summarised as follows:—

1. With subpassage, Negri bodies diminish in size until they become almost ultra-microscopic.

2. Of large size to begin with and staining brown to black, in the above experiment by the 6th passage they stain wholly black and are identical in staining reactions with the nucleolus.

3. There is a very decided drop in the numbers present at the 7th passage. The significance of this is not exactly clear. It is noteworthy, however, that the 7th passage is that in which the paralytic form of rabies is fully developed. In the 6th passage furious symptoms were still marked.

4. The diminution in the number of Negri bodies shown in the 7th passage is continued in the 8th and following passages.

5. The Negri bodies of the earlier passages (first three) showed a "veil" arrangement. Those of the later passages did not present this appearance, and may be described as "naked."

6. We regard the granules found in "veiled" Negri bodies as being composed of unaltered nucleolar substance.

7. The experiment given seems to show that the Negri body, as found in the cytoplasm of a ganglion cell, tends to disappear altogether from that region with the attainment of fixity of the virus. We have inoculated bullocks—the animals showing the largest Negri bodies known to us—one with street virus and another with fixed virus. The street virus brain showed large Negri bodies; the fixed virus brain showed none at all.

(435) Watson (E. M.). **The Negri Bodies in Rabies.**—*Jl. Experiment. Med.* 1913. Jan. Vol. 17. No. 1. pp. 29-42. With 2 plates.

It would appear that a large proportion of the observations recorded in this paper were made on material taken from the brains of two dogs each of which died twenty days after having shown symptoms suspicious of rabies. The bodies of the dogs after having been burnt were exhumed for examination.

The author supports the view that Negri bodies represent the actual cause of rabies, and claims to have obtained evidence that they are of a protozoal nature. His conclusions are as follows:—

1. The Negri bodies, as the etiological agent in rabies, present two general types or phases in morphology, in growth, and in reproduction.

2. These two phases are constantly cyclic in their development and correspond (1) to a multiplicative, or schizogonous, and (2) to a reproductive, or sporogonous, life cycle.

3. By the detailed study of these forms and their succeeding stages we are inclined to believe that the Negri bodies are definite protozoan parasites, and from a study of their life history we are led to place them in the suborder of Cryptocystes, or Microsporidia, of the Sporozoa, and more definitely among the Oligosporogenea of the Glugeidae family, which forms produce but one pansporoblast.

(436) Mackie (F. P.). **An Improved Method for Staining "Negri Bodies."**—*Indian Med. Gaz.* 1913. Jan. Vol. 48. No. 1. p. 20.

The following are the steps in the process:—

1. If the tissue has been fixed in Zenker's fluid the section must be treated with an alcoholic solution of iodine after the removal of the paraffin.
2. Wash with rectified spirit.
3. Flood the specimen with the following solution:

Alcoholic eosin	0·5 g.
50 per cent. alcohol	100 cc.

4. Set fire to the solution on the slide and allow it to burn out, thus leaving a strong watery solution of the eosin on the section. This should be allowed to act for a minute or two.
5. Wash and run on a little rectified spirit.
6. Treat the section until it assumes a pink colour (a few seconds) with rectified spirit, to every cubic centimetre of which one drop of one per cent. caustic soda in alcohol has been added.
7. Wash in rectified spirit to every cubic centimetre of which one drop of five per cent. acetic acid has been added.
8. Stain with haemalum or haematoxylin.
9. Dehydrate, clarify and mount in balsam.

When sections are stained by this method the following result is obtained:—

The groundwork has a blue or pink tint; the nerve cells are blue; the nucleoli are red or violet; the red blood corpuscles bright red, and the Negri bodies vermilion or magenta.

The main idea of the method was obtained from Muir.

The author suggests that the strong affinity possessed by Negri bodies for eosin indicates that the action of the dye upon the living virus might be tested. He has carried out a few inconclusive experiments himself.

UNDULANT FEVER.

(437) Ronchèse (A.). **Sur le Séro-diagnostic de la Mélitococcie avec des Cultures tuées par le Formol.** [The Serum Diagnosis of Undulant Fever with Cultures killed with Formalin.]—*Compt. Rend. Soc. Biol.* 1913. Feb. 7. Vol. 74. No. 5. pp. 210-212.

The experiments described in this paper were undertaken with the object of ascertaining whether cultures which have been preserved for a long period with formalin can be used for the agglutination test, or whether it is necessary to use freshly killed cultures.

The suspensions had added to them at the time of preparation two drops of formalin for every 15 cc.

In a series of tests the serum of persons who had recovered from the disease some months previously, and whose serum caused agglutination in a dilution of 1 in 100 was tested with (1) a fresh three-days-old culture of the micrococcus on agar, (2) an

emulsion made from a culture of the same age two months previously to which formalin had been added, (3) a similar suspension prepared a year previously.

It was found that the formalined suspensions were agglutinated to the same degree as the fresh one, and, further, that agglutination occurred more rapidly in the former than in the latter, the agglutination being the more rapid the older the emulsion.

Thus, the serum caused complete agglutination in the suspension of the fresh culture in dilutions of 1 in 10 and 1 in 50 in 40 minutes, and in a dilution of 1 in 100 in three hours. The periods necessary for complete agglutination with the emulsion prepared and formalined two months previously in the same dilutions were 15 minutes, 15 minutes, and 2 hours. With the emulsion prepared a year previously the periods were 5 minutes, 5 minutes, and 20 minutes respectively.

Tests were made with sera from people not infected with the organism, and it was found that there was no agglutination in dilutions of 1 in 10 even after three hours.

Heating the serum to 56° C. does not destroy its agglutinin either for the fresh emulsion or for formalined emulsions, but agglutination occurs a little more slowly.

(438) Vallet & Rimbaud (L.). **Étude expérimentale de l'Agglutination du** *Micrococcus melitensis.*—*Compt. Rend. Soc. Biol.* 1913. Feb. 24. Vol. 74. No. 7. pp. 323-324.

In this paper are briefly summarised experiments carried out to investigate the following points:—

I. The agglutination of the micrococcus by normal sera.

II. The effect of other infections upon the agglutination.

III. The agglutination produced by the sera of immunised animals.

IV. Cross agglutinations with four different strains of the micrococcus.

I. The sera of healthy guinea-pigs as a rule agglutinate the organism in low dilutions only—1 in 20.

The serum of the rabbit while still possessing a feeble agglutinating power for the organism causes agglutination in slightly higher dilutions than guinea-pig serum.

The serum of normal dogs has a very high agglutinating power for the *Micrococcus melitensis*. The author tested the serum of 21 dogs and in 18 instances there was agglutination in dilutions varying from 1 in 20 to 1 in 400, and even 1 in 600.

Heating the serum to 56° C., as suggested by Negre and Raynaud, has constantly destroyed the agglutinating power.

The addition of fresh serum to heated serum does not restore the agglutinating power.

II. The sera of animals inoculated with *B. eberthi*, *B. coli*, staphylococci, and other organisms have been used, but in no case was any agglutination observed.

III. The intravenous inoculation of rabbits and dogs with cultures of the micrococcus killed by heat at 60° C. confers a

very high power of agglutinating upon the sera, or increases that already present to a marked extent. Positive results may be obtained in dilutions from 1 in 1,000 to 1 in 3,000.

In the rabbit and the guinea-pig subcutaneous and intraperitoneal inoculations have a less marked effect.

Heating the serum to 56° C. may cause complete disappearance of the agglutinin. It caused complete disappearance of the agglutinin in every one of the guinea-pigs, in one out of five rabbits, and in three out of four dogs, even when the sera caused agglutination in dilutions of 1 in 1,000.

In cases in which heating does not absolutely destroy the agglutinin the limit of positive reaction is very much lowered.

IV. The agglutinability of various strains with normal sera shows very great variation. Some sera which will agglutinate certain strains in dilutions up to 1 in 600 fail to produce any agglutination whatever with other strains.

The serum of an immunised animal as a rule agglutinates other strains, but this is not invariably the case.

In a single series of cross agglutination tests the strains which are most highly agglutinised are not of necessity those which have been used for the immunisation of the animals.

When heating does not destroy the agglutinating power the agglutination indexes of the different strains before and after heating are not proportional.

MISCELLANEOUS.

(439) von Prowazek (S.). **Untersuchungen über die Tona der Pferde auf Samoa.** [Tona in Horses in Samoa.]—*Arch. f. Schiffs- u. Trop.-Hyg.* 1913. Jan. Vol. 17. No. 1. pp. 1-8. With 4 text-figures.

In Samoa horses are frequently the subject of granulating ulcers below the eyes and somewhat large granulating tumours which for the most part occur in the region of the fetlock and to which the term "Tona" is applied. The same term is used to designate framboesia in man.

Kramer and Hengeler, and Franke do not think that there is any connection between the disease in horses and the condition affecting the human subject. The affection of the horse results, in their view, from the neglect of wounds caused by thorns.

The present author states that the two diseased conditions are in reality quite distinct. In primary lesions in man typical framboesia spirochaetes can be demonstrated, but this is not the case with the equine disease.

The disease occurring in the neighbourhood of the eye differs from that involving the leg in that it usually takes the form of a deep ulcer not unlike "ulcus tropicum." The ulcers are crater-like and covered with a dirty brown, grey, or blood-stained material.

In sections pyogenic cocci are principally found, and the lesion is due to ordinary injuries in which the healing processes are delayed.

The lesion occurring on the leg is, however, of quite a different nature. This takes the form of a granulating tumour varying in size up to that of an apple and in many cases presenting a papillomatus appearance.

Microscopic examination shows that in these lesions there is marked development of the papillae of the cutis, associated with hyperkeratosis. In the basal layer of the epithelium there is active proliferation. In many cases a secondary lesion in the form of a crop of pustules occurs a round the periphery of the growth. In the superficial parts numbers of bacteria and cocci can be seen, but in the deeper layers fusiform bacilli and, still deeper, large numbers of spirochaetes, some of which actually appear to be intracellular, can be detected.

The author refers at some length to the various systems of nomenclature that have been adopted by different authors for the designation of the parasites generally referred to under the name "spirochaetes" and concludes that the most correct name is "*Borrelia*," although the most descriptive is "*Spirosoma*" (Schilling).

Since in the group of skin diseases at least three types of Borreliae occur, viz., large, intermediate, and small he suggests that the three forms occurring in "Tona" in the horse should be termed B. tonae magna, media, and minima.

Many observers consider that "tona" in the horse is due to systemic disease, while others consider it to be a localised disease of the foot which disappears when the animal is removed to a higher locality. This rather connects the condition with injuries caused by thorns, etc.

The author failed to transmit the condition experimentally in one instance.

In the deep layers of the epithelium eosinophilic cell-inclusions can be seen and these may be considered as parasites, but they are very scanty and the granules may be looked upon as nuclear fragments. The spirochaetes play a secondary part, penetrating into the deeper layers, while the bacteria remain in the more superficial portions of the growth. The spirochaetes may convert the tumour-like growth into a lesion resembling ulcus tropicum.

The spirochaetes may be made to disappear by frequent painting of the lesion with 20 per cent. eosin, the animal being kept in the sun. Smearing with precipitate ointment or painting with silver nitrate has the same effect.

(440) Carter (H. F.) & Blacklock (B.). **External Myiasis in a Monkey.**—*Brit. Med. Jl.* 1913. Jan. 11. p. 72.

The authors observed two small groups of dipterous larvae upon a monkey (*Cercopithecus callitrichus*) which was the subject of acute tuberculosis and died on the day after the observation was made. One batch was found in the nostril and on the side of the face, and the other on the side of the body near the groin.

In both batches there were larvae at different stages of development indicating that they had not all been deposited at the same time. In all 21 larvae were removed. Sixteen of them were of the muscid type and the remainder were specimens of *Fannia canicularis*. From the muscid larvae *Calliphora erythrocephala* and *Muscina stabulans* were obtained.

(441) von Ratz (S.). **Fütterungsversuche mit dem Virus der infektiöse Bulbärparalyse.** [Feeding Experiments with the Virus of Infectious Bulbar Paralysis.]—*Zeitschr. f. Infektionskrankh. Parasit. Krankht. u. Hygiene d. Haust.* 1913. Vol. 13. No. 1-2. pp. 1-7.

In the first series of experiments, brief details of which are given, nine mice were fed with brain tissue derived from cats or rabbits dead of the disease. Six of these animals became infected and died, death taking place on the 2nd to the 15th day. Nervous tissue from three of these mice was used for the inoculation of control rabbits, two of which died with typical symptoms.

In mice no characteristic symptoms are observed, but there was some paralysis of the hind legs.

Positive results were obtained with two white rats, the animals dying on the 17th and 18th days, and rabbits were successfully inoculated from them.

Feeding experiments with rabbits and guinea-pigs failed even though some of the rabbits were first treated with a 1 per cent. solution of sulphuric acid in order to produce intestinal catarrh.

Of 11 cats and dogs fed with infective material five became infected, and the symptoms produced correspond exactly with those observed in natural cases. As in the experiments with rabbits, some of the cats were previously given dilute sulphuric acid.

The author states that his experiments do not furnish absolute proof that the virus can be absorbed through sound mucous membranes, but he believes that such absorption is possible and quotes in support of this view the successful experiments of Galtier, Hogyes, Remlinger, and Conte with the virus of rabies.

(442) Balfour (A.). **Nasal Lesions in Glanders and Epizootic Lymphangitis.**—*Bull. Soc. Path. Exot.* 1913. Mar. Vol. 6. No. 3. pp. 145-146.

After referring to Bridé's report of the occurrence of nasal lesions in these diseases, the author states that he saw a mule at Mongalla which presented symptoms suggestive of glanders. There was a large swelling in the nasal region which was discharging thick pus, and there was a purulent discharge from the nose. There was no glandular enlargement. Microscopic examination of the pus revealed the presence of the *Saccharomyces farciminosus* in large numbers. Cultures showed no evidence of the presence of *B. mallei* No mallein was available. On examining the skull it was found that there had been extensive ulceration resulting in absorption of a large portion of the malar bone.

(443) Pettit (A.). **Procédé simple pour prélever du Sang chez les petits Rongeurs.** [Simple Method of obtaining Blood from small Rodents.]—*Compt. Rend. Soc. Biol.* 1913. Jan. 10. Vol. 74. No. 1. pp. 11-12.

The method described in this paper is said to be equally applicable to the guinea-pig, rat, and mouse.

A glass pipette which is drawn out to a moderately fine point is inserted at the inner canthus between the eye-ball and the wall of the orbit. It is passed along the wall of the orbit from before backwards, keeping it inclined at an angle of about 45 degrees, and directing it towards the optic nerve so as to perforate the cavernous sinus.

In the mouse the pipette requires to be passed in for a distance of about 5 millimetres, in the rat 10, and in the guinea-pig about 12.

As a rule the pressure of the blood is sufficient to force it up the pipette, but slight suction may be exerted by means of a rubber teat.

In order to lessen the risk of contamination of the blood some drops of an antiseptic may be instilled into the eye.

The method may be used when it is required to make intravenous inoculations into these animals.

RECENT LITERATURE.

[Continued from BULLETIN No. 3, pp. 193–196.]

Foot-and-Mouth Disease.

(444) BÖHM (J.). Zur Pathogenese der Maul- und Klauenseuche. [The Pathogenesis of Foot-and-Mouth Disease.]—*Zeitschr. f. Fleisch- und Milchhygiene*, 1913. Mar. 15. Vol. 23. No. 12, pp. 266–267.

(445) LOEFFLER (F.). Versuche über die Abtötung des Ansteckungstoffes der Maul- und Klauenseuche in vorschriftsmässig gepacktem Dünger. [Experiments in connection with the Destruction of the Virus of Foot-and-Mouth Disease in Dung.]—*Berl. Tierärzt Wochenschr.*, 1913. Feb. 13. Vol. 29, No. 7, pp. 113–115.

Leishmaniasis.

(446) GABBI (U.). Au Sujet de l'Historique du Kala-azar Méditerranéen.—*Bull. Soc. Path. Exot.*, 1913. Mar. Vol. 6. No. 3, pp. 141–143.

(447) LAVERAN (A.). Au Sujet de l'Historique du Kala-azar Méditerranéen.—*Bull. Soc. Path. Exot.*, 1913. Jan. Vol. 6. No. 1, pp. 23–24.

(448) MIGONE (L. E.). Un Cas de Kala-azar à Asuncion (Paraguay).—*Bull. Soc. Path. Exot.*, 1913. Feb. Vol. 6. No. 2, pp. 118–120.

Malaria.

(449) ZIEMANN (H.). Ueber die künstliche Weiterentwicklung (in vitro) des Tertian-Malariaparasiten. [The Artificial Cultivation of the Parasite of Tertian Malaria in vitro.]—*Deut. Med. Wochenschr.*, 1913. Feb. 6. Vol. 39. No. 6, p. 260.

Plague.

(450) GALLI-VALERIO (B.). *Bacterium pseudopestis murium* n. sp.—*Centralbl. f. Bakt.*, 1. Abt., Orig., 1913. Mar. 1. Vol. 68 No. 2, pp. 188–194. With 5 text-figures.

Rabies.

(451) MIESZNER (H.), KLIEM, & KAPFBERGER. Immunisierungsversuche gegen Tollwut. [Immunisation against Rabies.]—*Arch. f. Wissenschaft. u. Praktisch. Tierheilkunde*, 1913. Feb. Vol. 39. No. 3, pp. 169–209.

Spirochaetosis.

(452) NUTTALL (G. H. F.). The Herter Lectures. I. Spirochaetosis. Lecture delivered on the Herter Foundation, Johns Hopkins University, Baltimore, Maryland, U.S.A., Oct. 8, 1912.—*Parasitology*, 1913. Jan. Vol. 5. No. 4, pp. 262–274.

Trypanosomiasis.

(453) BRUMPT (E.). Evolution de *Trypanosoma lewisi, duttoni, nabiasi, blanchardi*, chez les Puces et les Punaises. Transmission par les Déjections. Comparaison avec *T. Cruzi*. [*T. lewisi, duttoni, nabiasi, blanchardi* in Fleas and Bugs. Transmission by means of Dejecta. Comparison with *T. Cruzi*.]—*Bull. Soc. Path. Exot.*, 1913. Mar. Vol. 6. No. 3, pp. 167–171.

(454) ——. Immunité partielle dans les Infections à *Trypanosoma cruzi*, Transmission de ce Trypanosome par *Cimex rotundatus*. Rôle régulateur des Hotes intermédiaires. Passage à travers le Peau.—*Bull. Soc. Path, Exot.*, 1913. Mar. Vol. 6. No. 3, pp. 172–176.

(455) Chatton (E.). Position Systématique et Signification Phylogénique des Trypanosomes Malpighiens des Muscides. Le genre *Rhynchoidomonas* Patton.—*Compt. Rend. Soc. Biol.*, 1913. Mar. 14. Vol. 74. No. 10, pp. 551–553.

(456) —— & Leger (M.). L'Autonomie des Trypanosomes propres aux Muscides démontrée par les élevages purs indéfinis.—*Compt. Rend. Soc. Biol.*, 1913. Mar. 14. Vol. 74. No. 10, pp. 549–551.

(457) Lazillo (V.). La Durina (Sifilide Equina) in Due Asini. [Dourine in two Asses.]—*Giorn. di Med. Vet.*, 1913. Jan. 18. Vol. 62. No. 3, pp. 45–49.

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